DOCUMENT RESUME

ED 134 426 SE 021 563

TITLE	Chemistry of Living Matter, Energy Capture & Growth, Parts Three & Four of an Integrated Science Sequence, Teacher's Guide, 1973 Edition.
INSTITUTION	Portland Project Committee, Oreg.
SPONS AGENCY	National Science Foundation, Washington, D.C.
PUB DATE	73
NOTE	235p.; For 1971 Edition Student Guide, see ED 064 097; For related Teacher's Guides, see SE 021 558-562
EDRS PRICE	MF-$0.83 HC-$12.71 Plus Postage.
DESCRIPTORS	*Energy; *Integrated Curriculum; Physical Sciences; *Science Activities; Science Course Improvement Project; Science Education; Science Units; Secondary Education; *Secondary School Science; *Unified Studies Programs; Units of Study (Subject Fields)
IDENTIFIERS	*Portland Project

ABSTRACT

This teacher's guide includes parts three and four of the four-part third year Portland Project, a three-year integrated secondary science curriculum sequence. The underlying intention of the third year is to study energy and its importance to life. Energy-related concepts considered in year one and two, and the concepts related to atomic structure and particle phenomena considered earlier in the third year are further built upon in this volume. Chapters include: monomers and how they are built; chemistry of simple carbon compounds: polymers or stringing monomers together; polymers in 3D or the shape of things to come; where the action is - the active site; polymers to polymers; genes, proteins, and mutations; energy capture; energy consumption and metabolism; and metabolism and genes. Notes to the teacher, examples of data, and problem calculations are included. (Author/SL)

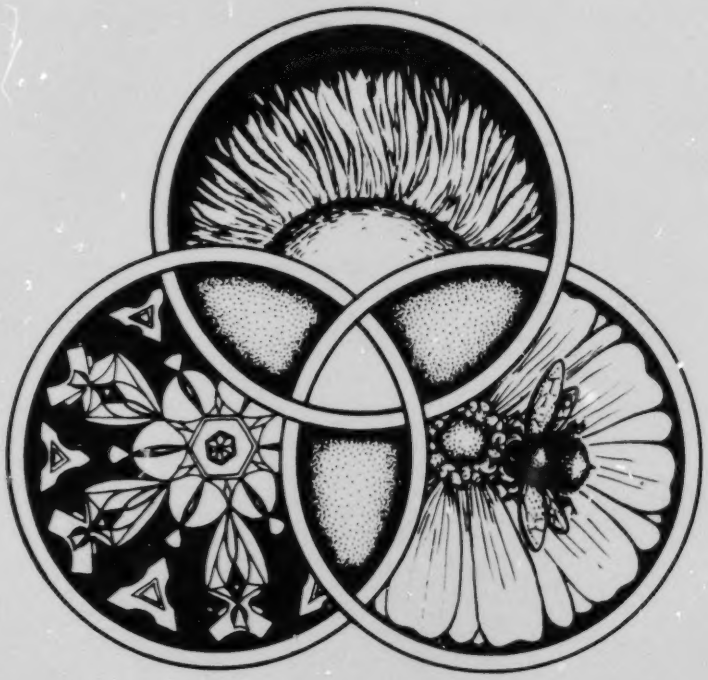
****
* Documents acquired by ERIC include many informal unpublished *
* materials not available from other sources. ERIC makes every effort *
* to obtain the best copy available. Nevertheless, items of marginal *
* reproducibility are often encountered and this affects the quality *
* of the microfiche and hardcopy reproductions ERIC makes available *
* via the ERIC Document Reproduction Service (EDRS). EDRS is not *
* responsible for the quality of the original document. Reproductions *
* supplied by EDRS are the best that can be made from the original. *
****

YEAR THREE

CHEMISTRY OF LIVING MATTER, ENERGY CAPTURE & GROWTH

PARTS THREE & FOUR
OF
AN INTEGRATED SCIENCE SEQUENCE

ED134426



U.S. DEPARTMENT OF HEALTH
EDUCATION & WELFARE
NATIONAL INSTITUTE OF
EDUCATION

THIS DOCUMENT HAS BEEN REPRODUCED EXACTLY AS RECEIVED FROM THE PERSON OR ORGANIZATION ORIGINATING IT. POINTS OF VIEW OR OPINIONS STATED DO NOT NECESSARILY REPRESENT OFFICIAL NATIONAL INSTITUTE OF EDUCATION POSITION OR POLICY.

D. Wilson

TEACHER GUIDE

1973 EDITION

prepared by
THE PORTLAND PROJECT COMMITTEE
under a grant from
THE NATIONAL SCIENCE FOUNDATION

SE 021 563

CHEMISTRY OF LIVING MATTER, ENERGY CAPTURE & GROWTH

AN INTEGRATED SCIENCE SEQUENCE

1973 EDITION

DIRECTOR:
Dr. Karl Dittmer
Portland State University
Portland, Oregon

CO-DIRECTOR:
Dr. Michael Fiasca
Portland State University
Portland, Oregon

© Copyright 1970
by Portland State University

TABLE OF CONTENTS

PART THREE: CHEMISTRY OF LIVING MATTER

Expanded Outline 4

Chapter I. Monomers and How They Are Built..... 8

Chapter II. Some Chemistry of Simple Carbon Compounds..... 42

Chapter III. Polymers or Stringing Monomers Together..... 60

Chapter IV. Polymers in 3D or the Shape of Things to Come.... 84

Chapter V. Where the Action Is-The Active Site..... 98

Chapter VI. Polymers to Polymers..... 106

Chapter VII. Genes, Proteins and Mutations..... 130

PART FOUR: ENERGY CAPTURE AND GROWTH

Expanded Outline..... 138

Chapter I. Energy Capture..... 140

Chapter II. Energy Consumption and Metabolism..... 160

Chapter III. Metabolism and Genes..... 180

Dedication

This volume is dedicated to the memory of Vernon Cheldelin under whose guidance and leadership integration of the sciences for Oregon secondary school youth was begun in 1963.

The Portland Project was initiated in the fall of 1962 when two secondary school teachers, one with background in CBA chemistry, the other having responsibility for PSSC physics, began to note and discuss the redundancy in their respective courses. Why should students be subjected to this repetitious and fragmented representation of the physical sciences? they asked. A Steering Committee met to pursue the problem further and perhaps enlist the support of a funding organization to permit its exploration in depth. Under the able and devoted leadership of Vernon Cheldelin, Dean of the School of Science at Oregon State University (deceased), two proposals prepared for support by the National Science Foundation were funded in the summers of 1963 and 1964.

Thirty-five scientists and teachers devoted various quantities of time as writers, consultants, pilot teachers, and evaluators, with the aim of ascertaining the feasibility and efficiency of the integration of chemistry and physics. Concurrently and subsequently, other groups in other parts of the country have carried on studies that are approximately parallel to this one.[1] Though the conceptual development and points of emphasis differ, the various groups are satisfied that integration of science courses is not only feasible but highly desirable.

Dr. Michael Fiasca of the Education and Science Staffs of Portland State University conducted an evaluation which revealed that subject matter achievement in chemistry and physics and critical thinking abilities are enhanced among students who studied the integrated courses over those who study the separate disciplines of

---

[1] Federation for Unified Science (FUSE) was recently organized to act as a clearinghouse of information on integrated science courses. Victor Showalter at Ohio University is the chairman of this committee.

chemistry and physics. It should be emphasized that though these differences were apparent, it could not be demonstrated that they were statistically significant.[2] A concomitant result showed that enrollments in the two-year integrated courses were dramatically greater than in the separate courses.

A survey completed April 16, 1967 showed that there were forty-four schools in twenty states using the Portland Project integrated chemistry-physics course.[3]

Mounting evidence in the literature from prominent persons working in science education strongly supported this mode of organization. Dr. Jerrold R. Zacharias, the prime instigator of the PSSC physics program, exemplified the changing attitude of scientists and educators:

> The division of science at the secondary school level, into biology, chemistry and physics is both unreasonable and uneconomical.
>
> Ideally, a three-year course that covered all three disciplines would be far more suitable than a sequence of courses which pretends to treat them as distinct. Today such a three-year course would be difficult to fit into the educational system, but much of this difficulty might be overcome at once if such a course existed, and it might well be that present tendencies in education would soon overcome the rest.
>
> In any case, a greater coordination of the three subjects is possible even within the existing framework. It is understandable that the groups which developed the existing programs, each of which faced great problems of its own as it worked toward its goals, were reluctant to embark on the larger task of giving coherence to the sum of their efforts. With the programs now complete or approaching completion, it may be that the time has arrived for this necessary step.[4]

---

[2] Detailed results of this study may be obtained by writing to Dr. Fiasca at Portland State University.

[3] Detailed enrollment figures and addresses of people who are using the Portland Project courses may also be obtained from Dr. Fiasca.

[4] From page 52 of Innovation and Experiment in Education, a Progress Report of the panel on Educational Research and Development to the U.S. Commissioner of Education, the Director of the National Science Foundation, and the Special Assistant to the President for Science and Technology, March, 1964.

Stimulated by the apparent success of their original work towards this kind of integrated course, persons close to the Portland Project began to discuss extension of their work to include biology with chemistry and physics in a three-year sequence. A third proposal was prepared in 1966 and granted support by the National Science Foundation. Dr. Arthur Scott, member of the Chemistry Department at Reed College who has had deep interest in the Portland Project since its inception, graciously offered his talents, energy and time to carry on the project after Dean Cheldelin's death.

A writing conference was conducted on the Portland State University campus during the summer of 1967 to develop materials such as teacher and student guides. Eight local pilot schools committed approximately five hundred students and twelve pilot teachers for testing and evaluation. Dr. Donald Stotler, Supervisor of Science for the Portland School District, has had an active part in this and other phases of this project.

Twenty-six persons whose functions were writing, consulting, analysis, and editing met on the Portland State campus beginning June 14, 1967 to begin preliminary work on the integrated course. Their first task was to formulate an outline that displayed logical content development utilizing concepts out of biology, chemistry and physics. Particular attention was paid to matching students' abilities, interest and maturity level with the sophistication of concepts as nearly as this was possible to do. Then the committee perused material developed by the national curriculum groups --PSSC, Project Physics, CBA, CHEMS, BSCS and IPS -- in search of material to implement the outline they constructed previously. In the absence of appropriate materials, major and minor writing projects were initiated.

The writing committee continued its work in the summers of 1968 and 1969 with Dr. Karl Dittmer, Dean of the Division of Science, as director. Four major projects were tackled and completed: (1) extensive revisions were effected in the three-year outline, (2) the first- and second-year courses were revised based upon

student and teacher feedback, (3) the third-year course was developed incorporating Harvard Project Physics materials as a main vehicle, and (4) an evaluation program for the three-year course was developed.

Working Committee

Carl Bachhuber - Physics
Portland State University
Portland, Oregon

Renee Bergman - Physical Science
Roosevelt High School
Portland, Oregon

Louis Bixby - Chemistry
St. Louis Country Day School
St. Louis, Missouri

Howard Browning - Chemistry and Physics
Benson Polytechnic School
Portland, Oregon

David Cox - Chemistry and Physics
Rex Putnam High School
Milwaukie, Oregon

William Devery - Psychology
Reed College
Portland, Oregon

Steven Dimeo - Editor
University of Oregon
Eugene, Oregon

Karl Dittmer - Co-Director and Biochemistry
Portland State University
Portland, Oregon

Arleigh Dodson - Chemistry
Lewis and Clark College
Portland, Oregon

Len Dooley - Biology and Chemistry
Sunset High School
Beaverton, Oregon

Ray Ellickson - Physics
University of Oregon
Eugene, Oregon

Michael Fiasca - Co-Director, Physics
Portland State University
Portland, Oregon

Edward Fuller - Chemistry
Beloit College
Beloit, Wisconsin

Curtis Guthrie - Chemistry
Wilson High School
Portland, Oregon

Jean Halling - Chemistry
Beaverton High School
Beaverton, Oregon

Glen Hampshire - Chemistry and Physics
Jefferson High School
Portland, Oregon

Lois Helton - Editor
Beaverton School District #48
Beaverton, Oregon

Marshall Herron - Physics
Portland State University
Portland, Oregon

George Ingebo - Director of Research
Portland School District
Portland, Oregon

Lloyd Ingraham - Physics
Grant High School
Portland, Oregon

Henry Kilmer - Biology and Chemistry
Rex Putnam High School
Milwaukie, Oregon

Helen Koopman - Physical Science
Jefferson High School
Portland, Oregon

Edward Kormondy - Director, Comm. on Undergraduate Educ. in the Biological Sciences
Washington, D. C.

Alfred Levinson - Chemistry
Portland State University
Portland, Oregon

David Malcolm - Biology
Portland State University
Portland, Oregon

Edmund McCollough - Chemistry
Cleveland High School
Portland, Oregon

Jack McGoldrick - Chemistry
Rex Putnam High School
Milwaukie, Oregon

Lloyd Meskimen - Physics
Roosevelt High School
Portland, Oregon

Thomas Miles - Biology
Cleveland High School
Portland, Oregon

John Myers - Biology
Portland State University
Portland, Oregon

Leon Pape - Biophysics
California State College at Los Angeles
Los Angeles, California

Arnold Pickar - Physics
Portland State University
Portland, Oregon

Clarence Porter - General Science
Portland State University
Portland, Oregon

David Porter - Physics
Portland Community College
Portland, Oregon

Jack Sadler - Biophysics
University of Colorado Medical Center
Denver, Colorado

Guenter Schwartz - Physics
Florida State University
Tallahassee, Florida

Arthur Scott - Chemistry
Reed College
Portland, Oregon

Joseph Sklenicka - Physics
Franklin High School
Portland, Oregon

Dean Smith - Chemistry
Beaverton High School
Beaverton, Oregon

Donald Stotler - Supervisor of Science
Portland School District
Portland, Oregon

Harold Wik - Science Coordinator
Beaverton School District #48
Beaverton, Oregon

John H. Wirtz - General Science
Portland State University
Portland, Oregon

Pilot Schools

The following schools have served as pilot schools for the pilot course during one or more of the past three academic years.

Adams High School
Portland, Oregon
- Glen Hampshire
- Lloyd Meskimen
- Thomas Miles

Aloha High School
Aloha, Oregon
- Mary Lou Combs
- Elvis Dellinger
- Nelson Doeleman
- Ted Parker

Beaverton High School
- Roger Berg
- Jean Halling
- Lois Helton
- H. Dean Smith

Benson Polytechnic School
- Howard Browning
- W. B. Chase
- Robert Franz
- W. L. Hoffman

Central Catholic High School
Portland, Oregon
- Jacob A. Mosbrucker
- Peter Roerig

Cleveland High School
Portland, Oregon
- John Brown
- Edmund McCollough

Franklin High School
Portland, Oregon
- John Neeley
- Joseph Sklenicka

Grant High School
Portland, Oregon
- Myra N. Rose

Jefferson High School
Portland, Oregon
- Ronald Kawamoto
- Leslie Morehead
- Kenneth Starbuck

Parkrose High School
Parkrose, Oregon
- Donald Pearson

Rex Putnam High School
Milwaukie, Oregon
- Dennis Axness
- David Cox
- Jerry Fenton
- Henry Kilmer
- Jack McGoldrick

Roosevelt High School
Portland, Oregon
Renee Bergman
Kenneth Fuller
Sue Storms

Sunset High School
Beaverton, Oregon
Leonard M. Dooley

Wilson High School
Portland, Oregon
Curtis D. Guthrie
Norman Sipple

Woodland High School
Woodland, Washington
Donald G. Fry
George L. Stroud

ACKNOWLEDGEMENTS

Linda Anderson - Receptionist

Cecelia Benner - Secretary

Judy Bierman - Secretary

Doni Budlong - Secretary

Alice Eyman - Secretary

Mary Grant - Artist

Ethel Hays - Secretary

Lothar Graudins - Artist

Neomia Kendrix - Technician

Peggy Munyer - Typist

Janice Page - Laboratory Assistant

Robert Poon - Laboratory Assistant

Dianne Poppino - Laboratory Assistant

Jean Sims - Secretary

Linda Tham - Laboratory Assistant

Jill Timmons - Secretary

Kathryn Ann Torvik - Artist

Shirley Tsang - Laboratory Assistant

Barbara Wiegele - Secretary

STUDENT PARTICIPANTS

Patrick Moore - Jefferson High School (sow bug experiment)

The decision to try to develop a three-year integrated science course which would replace the traditional three courses in biology, chemistry and physics is based on several considerations. Among them are:

(1) a conviction that modern developments have made the division of science under these three headings obsolete;

(2) a recognition that the traditional courses overlap in many areas, resulting in a great deal of duplication and repetition as in the gas laws, atomic and nuclear structure, calorimetry and the kinetic molecular theory;

(3) a feeling that terminal students, who take no more than one year of science, deserve to get a taste of all of science rather than just one aspect, as they do in the conventional programs; and

(4) a desire to emphasize the unity in the approach to natural phenomena and the similarity in the methods, techniques and apparatus used by scientists in all fields.

A natural question arises as to what distinguishes this course from a general science course expanded to three years. The answer is that this course does not consist of a number of unrelated topics that might be taken up in any order; rather, it treats science as a structure that proceeds from observation to the development of general principles and then to the application of those principles to more involved problems. The emphasis in a general science course is on the results of science; the emphasis here is on the methods and reasoning by which scientists have arrived at these results.

The three-year course outline shows that a number of topics such as properties of matter, energy, heat, and certain biological concepts are discussed at the first-year level and again later in the course. This re-cycling is deliberate. It is intended to introduce students in a semi-quantitative way to some of the

significant generalizations of science and to show how these generalizations arose. These topics are treated again in the second and third years when greater facility with mathematics on the part of students makes it possible for them to understand and appreciate discussions of these topics in the succinct and precise language of mathematics.

An excessively formal and quantitative approach is avoided in the first year for several reasons. Students at this level do not extract essential meaning from such a presentation of information; furthermore, first encounters with new ideas should proceed from an intuitive, non-quantitative confrontation to one that is more quantitative. Teachers have spoken out against teaching and learning methods which substitute equations, formulas and other quantitative representations for first-hand experience, word descriptions, examples and illustrations. These criticisms are just as valid for students who are very capable and very interested in science as they are for other students. Moreover, the mathematical sophistication of students at this level is such that they are unable to follow most mathematical arguments as explanations for natural phenomena.

The typical science experiences of most secondary school students consists of one or two years devoted to general science and biology. Few study physics and chemistry. A significant advantage to the course of study described here is that students are given a chance to study physics and chemistry at a level of rigor that is consistent with their ability and their mathematical maturity. Students who terminate their study of science at the end of one year get a significant exposure to the structure of biology, chemistry and physics as they are presented in the latest curricular developments. Students who might not elect science beyond the first year because of lack of interest in biology may be attracted by the chemistry or physics portions of the course and elect to take an additional year or two of science. Students who are "turned on" by biology may wish to pursue further study of biochemical topics in Years II and III.

After considering these problems and goals, the general course outline for the first year of the course was derived. It consists of four main parts:

(1) Perception and Quantification

(2) Heat, Energy and Order

(3) Mice and Men

(4) Environmental Balance?

The year begins with a study of the perceiver, moves on to the perceived, and ends with the interaction of the perceiver with the perceived. The first-year student starts out by gaining a better awareness of the nature of his perception and senses -- the faculties that make him aware of the world around him. With an increased understanding of these perceptual abilities, he can turn to the environment and then relate himself to it. He finds that his perception is limited and that he often needs to call on technological and conceptual extensions and that even these have their limitations.

The importance of organization and classification as parts of perception is emphasized. The physical properties of matter are introduced and studied as aids in organization and classification of chemicals. The identification of unknowns by study of their physical properties and use of organized data on punch cards is the culminating experiment of the Perception unit.

Apart from the great diversity exhibited in nature, which the scientist must organize in order to comprehend, certain unifying principles are essential for deeper understanding. The most powerful of these is the energy concept, which is explored in the "Heat, Energy and Order" unit in several of its ramifications - physical, chemical and biological. The discussion begins by developing an experientially important energy form, viz., heat. The macroscopic aspects of heat as embodied in calorimetry are related to the microscopic in terms of random molecular motion. This builds confidence in the idea of the atomic nature of matter, which

xx is essential to much of the unit. Various energy conversions form the vehicle for extending and generalizing the energy concept. Nuclear energy is developed in sufficient detail to underscore its environmental and social significance. Finally, the thermodynamic limitations and implications of energy conversion are explored, ending with a view of life as a supremely artful organizer in nature, a mechanism powered by energy which creates wondrous "local order", but always at the expense of influencing its environment.

The growth of a mouse colony carries the thread of the unit "Mice and Men." As the colony develops, students learn many things about the concept of population. The food and water consumed and products eliminated tie the mouse colony back to the unit "Heat, Energy and Order", and point ahead to the chapter on communities and to the unit "Environmental Balance?".

The cell concept is given prime position in this unit. It is used to enter topics on reproduction, embryology and maturation which are observed in the mice and other organisms. The mice selected for the original colony are such that an experiment in Mendelian genetics comes out of the observations students make as the colony develops. In most of the chapters man is an important organism and receives as much attention as the mouse, although the data are often secondhand.

A rather unpleasant fact that must be faced is that as our population increases, and human activities are directed towards increasing the standard of living for this population, strains are placed upon the environment. As students discover in "Mice and Men," the size of the community has a relation to both the quantity of the food, water and energy required and the quantity of waste products produced. To develop the concept of a closed system and point out the necessity for environmental management, an analogy between the earth and a spaceship is made. Students are then introduced by a multi-media approach to the nature of some of our common pollutants (with emphasis upon air, water, heat, noise and radiation)

as well as their effects. Following this students are encouraged to undertake a rather detailed study of a particular type or aspect of pollution. Emphasis here is placed upon student activity, which may take any number of forms. The culminating activity centers around discussion of these special studies together with the complex relations involved within the environment. It is hoped that out of these studies students will become aware of threats which exist to man's future on this planet.

THE SECOND YEAR COURSE

The second year of the course is considerably more quantitative in its approach than the first. This is the case because (1) the students are one more year along in their mathematical preparation, (2) the students who elect to take a second year of science are more likely to exert the effort to master more difficult topics, and (3) many of the quantitative aspects of physics and chemistry are basic to an understanding of molecular biology, which is an important part of the following year's work.

The second year consists of two parts:

(1) Motion and Energy

(2) Chemical Reactions

Year II begins with the study of motion, going from the quantitative description of motion to a consideration of what causes motion and a discussion of Newton's laws. There follows the development of the laws of conservation of momentum and energy, including a discussion of energy in biological systems. This section, which is primarily mechanics, culminates with a discussion of kinetic molecular theory.

Due to recent advances in both molecular biology and biochemistry, the descriptive approach to biology has gradually given way to one that is primarily analytical. It is now necessary, even on the high school level, for the serious biology student to have a more thorough understanding of those concepts normally

xxii embodied in the "modern" high school physics and chemistry courses. The major objective of "Chemical Reactions" is to build some of those basic chemical concepts that are necessary for an analytical study of "The Chemistry Of Living Matter" and "Energy Capture and Growth."

The following subtopics of this section help in the realization of the major objective: Some of the topics discussed are the mole concept, equation writing, energetics associated with chemical reactions, the dynamic nature of particles and their interactions and the application of energy and equilibrium to chemical systems.

THE THIRD YEAR COURSE

Year III consists of four parts:

(1) Waves and Particles

(2) The Orbital Atom

(3) Chemistry of Living Matter

(4) Energy Capture and Growth

The underlying rationale of the third year is a study of energy and its importance to life. The first thrust is to build the orbital model of the atom using, as background, waves, electromagnetism and historical models of the atom. Once the orbital model is established as a representation of the localization and directionalization of electronic energy, structural models are built to show how biopolymers are spatially arranged and experiments are done to give evidence of energy relationships. With shape, size and energy relationships of molecules established, the DNA molecule is introduced. The culmination of this work comes in the final section when photosynthesis is considered. With this topic, much that has gone before is brought to a logical focus.

These topics are most appropriately placed in the third year of the integrated sequence after students have developed some facility with basic ideas

from chemistry and physics - e.g., quantitative knowledge about energy, mechanism of chemical reaction, equilibrium, rate of reaction, the photon and wave nature of light, electrical phenomena, and kinetic molecular theory. They should not now simply parrot biochemical processes such as photosynthesis and cell respiration but should understand the many chemical and physical principles which underlie these processes.

Time is allotted at the conclusion of Year III for individual investigation and studies.

Three-Year Course Outline

	TOPIC	REFERENCE
	First Year	
Part One:	Perception and Quantification	
	I. Sensing and Perceiving	PP*
	II. Measurement, Distribution, Organization and Communication	PP
Part Two:	Heat, Energy and Order	
	I. Heat	PP
	II. Temperature and Chaos	PP
	III. Energy	PP
	IV. Nuclear Energy and Radioactivity	PP
	V. Trends in Nature	PP
Part Three:	Mice and Men	
	I. Reproduction and Development	PP
	II. Genetics	PP
	III. Genetics and Change	PP
	IV. Populations	PP
	V. Ecology	PP
Part Four:	Environmental Balance?	PP

* PP designation signifies materials produced by the Portland Project.

TOPIC		REFERENCE
	Second Year	
Part One:	Motion and Energy	
I.	Motion	HP*
II.	Newton Explains	HP
III.	Multi-Dimensional Motion	HP
IV.	Conservation	HP
V.	Energy - Work	HP
VI.	Kinetic Theory of Gases	HP
Part Two:	Chemical Reactions	
I.	The Mole as a Counting Unit	PP
II.	Combinations of Gases	PP
III.	A Useful Form of P=kDT	PP
IV.	Chemical Equations	PP
V.	Electrical Nature of Matter	CHEMS+
VI.	Basic Particles	CHEMS
VII.	Energy Effects in Chemical Reactions	CHEMS
VIII.	Rates of Reactions	CHEMS
IX.	Equilibrium	CHEMS
X.	Solubility	CHEMS
XI.	Acid-Base	CHEMS
XII.	Oxidation-Reduction	CHEMS

---

* HP designates Harvard Project Physics material.
\+ CHEMS designates material derived from the Chemical Educational Materials Study.

	TOPIC	REFERENCES
	XIII. Stoichiometry	CHEMS
	Year Three	
Part One:	Waves and Particles	
	I. Waves	HP
	II. Light	HP
	III. Electricity and Magnetic Fields	HP
	IV. Faraday and the Electrical Age	HP
	V. Electromagnetic Radiation	HP
	VI. The Chemical Basis of Atomic Theory	HP
	VII. Electrons and Quanta	HP
	VIII. The Rutherford-Bohr Model of the Atom	HP
	IX. Some Ideas from Modern Physical Theories	HP
Part Two:	The Orbital Atom	
	I. Atoms in Three Dimensions	PP
	II. Many-Electron Atoms	CHEMS
	III. Ionization Energy and the Periodic Table	CHEMS
	IV. Molecules in the Gas Phase	CHEMS
	V. The Bonding in Solids and Liquids	CHEMS
Part Three:	The Chemistry of Living Matter	
	I. Monomers and How They Are Built	PP
	II. Polymers or Stringing Monomers Together	PP
	III. Polymers in 3-D or The Shape of Things to Come	PP
	IV. Where the Action Is--The Active Site	PP

V. How Polymers Make Polymers PP

VI. Genes, Proteins and Mutations PP

Part Four: Energy Capture and Growth

I. Energy Capture PP

II. Energy Consumption - Metabolism PP

III. Metabolism and Genes PP

CHEMISTRY OF LIVING MATTER

Outline: Chemistry of Living Matter

SECTION	TOPIC	PAGE
Chapter I	Monomers and How They Are Built	8
A.1	What You Will be Doing	8
A.2	The Kit	8
A.2.a	Metal Parts	9
A.3	Hybrid Orbitals	10
A.4	Single Bonds	12
A.5	Double Bonds	12
A.6	Triple Bonds	15
A.7	Plastic Tubing	15
A.8	Construction of Bonds	17
A.9	Bonds Between Unlike Atoms	18
A.9.a	To Build a C-O Bond	18
A.10	Enough Talk; Let's Build	19
A.11	Words about Molecules	21
A.12	Building Multiple Bonds	24
A.13	Ring Structure	31
B.	Functional Groups	33
B.1	Alcohols	33
B.2	Other Reactive Groups	33
B.3	Alkyl Radicals	34
B.4	Amines	35
B.5	Models of Reactions	35
B.6	Functional Groups--Nomenclature	35a

SECTION	TOPIC	PAGE
Chapter II	Some Chemistry of Simple Carbon Compounds	42
Introduction		42
A.	Origins of Methane	43
A.1	Primordial Methane	43
A.2	Methane of Biological Origin	44
A.3	Methane from Petroleum	45
A.3.a	Petroleum Derivative Products	47
A.4	Natural Gas	50
B.	Uses of Methane	53
B.1	Energy Sources	53
B.2	Chemical Intermediate	54
B.2.a	Carbon Black	54
B.2.b	Methanol	56
B.2.c	Other Chemicals	58
Chapter III	Polymers or Stringing Monomers Together	60
A.1	Building Models of Monomers and Polymers	60
A.1.a	Experiment: Preparation of a Polyamide-Nylon	64
A.2	Proteins, Nucleic Acids, Carbohydrates	64
A.2.a	Experiment: Polymer Size	74
A.3	A Closer Look at Size	75
A.3.a	Experiment: Polymer Size--Quantitative	76
Chapter IV	Polymers in 3D or the Shape of Things to Come	84
A.	Polymer Shape	84
A.1	Experiment: Polymer Shape or Permanent Wave	86
B.	Weaker Bonds	87

SECTION	TOPIC	PAGE
B.1	H-Bonds Between Bases	87
B.2	Hydrophobic Bonds Between Bases	91
C.	The Importance of Shape	93
Chapter V	Where the Action Is-The Active Site	98
A.	Lab Activities With Enzymes	98
A.1	Experiment: Proteolytic Enzymes	98
A.2	Saliva and Its Substrate	99
A.3	Specificity of Urease	100
Chapter VI	Polymers to Polymers	106
A.	The Activation Problem	107
A.1	Experiment: Aspirin Synthesis	108
A.2	Experiment: The Synthesis and Breakdown of Starch by Phosphorylase	111
B.	The Sequence Problem or How Cells Make RNA	114
C.	The Sequence Problem Continued: How Cells Make Protein	118
D.	The Sequence Problem Completed or the Master Molecule	124
E.	Molecular Symbiosis	127
Chapter VII	Genes, Proteins and Mutations	130
A.	Mistakes in Genetic Material	130
B.	Effects of Mistakes	131
C.	Causing Mistakes	132

OPTIONAL

Some introduction to organic chemistry has been found to be helpful. Chapter 18 of Chem Study, Chapters 20 and 21 of Chemistry, A Modern Course, Smoot, Price, Barrett, or a comparable reference might be acceptable. There are some excellent paperbacks available.

SUGGESTED READING

Really indispensable:

1. Arditti, J. and A. Dunn, Experimental Plant Physiology - Experiments in Cellular and Plant Physiology, New York, Holt, Rinehart and Winston, 1969. Paperback - Excellent "how to" guide for the teacher.

2. Readings from Scientific American, The Molecular Basis of Life - an Introduction to Molecular Biology, San Francisco, W. H. Freeman and Company, 1968. Paperback.

Fine for background:

3. Asimov, I., The Genetic Code, New York, New American Library, Inc., 1962. Paperback. (S)

4. Asimov, I., Life and Energy - An Introduction to the Physical and Chemical Basis of Modern Biology, New York, Bantam Books, 1965. Paperback.

5. Bernhard, S. A., Structure and Functions of Enzymes, New York, W. A. Benjamin, Inc.

6. Eliel, E. L. et al, Conformational Analysis, New York, John Wiley and Sons. Paperback.

7. Geis, I., and R. E. Dickenson, Protein Structure in 3 D. Paperback.

8. Lehninger, A. L., Bioenergetics, New York, W. A. Benjamin, Inc., 1965.

9. McElroy and Swanson, Modern Cellular Biology, New Jersey, Prentice-Hall. (S)

10. McKusick, W., Human Genetics. New Jersey, Prentice-Hall. Paperback.

11. Sanderson, R. T., Teaching Chemistry with Models. New York, Van Nostrand-Reinhold Books.

12. Watson, J. D., Double Helix. New York, New American Library, Inc., 1968. Paperback.

13. Watson, J. D., Molecular Biology of the Gene. New York, W.A. Benjamin, Inc. Paperback.

14. A Scientific American Book, The Physics and Chemistry of Life. New York, Simon and Schuster, 1955. Paperback (S).

15. Nomenclature of Organic Chemistry. New York, Plenum Publishing Corp. Paperback.

(S) Suitable for use by student.

TEXT SECTION	ROUGH TIME ESTI-MATES	EXPERIMENTS	DEMONSTRATIONS	TEACHING AIDS	OTHER STUDENT ACTIVITIES	PROBLEMS	OUTSIDE READING
Chap. 1 Monomers and how they are built	↑			Framework Molecular Models Kit		1, 2, 4, 5 6, 12, 13	CHEMS 13-1 Sources of carbon compounds CHEMS 13-2 Mol. structure of carbon compounds
B.2 Let's build	1 week				Model-building, single bonds	7, 8, 9	
C. Double bonds and more C.1 Ring structures	↓				Model building Model-building	14,	
D. Functional groups					Model-building		

Chapter I: MONOMERS AND HOW THEY ARE BUILT

A.1 WHAT YOU WILL BE DOING

Your studies in Chemistry of Living Matter will be a start at looking into fundamental biological processes. You will read about and discuss molecular rearrangements and energy interchanges which are bioprocesses. You will investigate these processes in two ways. Since the actual shape of a molecule is important, you will start by building molecular models. Then, you will do experiments involving the buildup, breakdown and energy transfer of these biomolecules. As much as possible, model building of similar molecules will go along with the lab exercises. In this way you will be able to study the interrelationship of structure and reaction.

A.2 THE KIT

Balloons may be used as demonstration aids and for hybrid orbitals.

For the model building section, you will use the "Framework Molecular Model" kit. This kit will enable you to build a model with reasonably accurate bond lengths, bond angles and bond thicknesses. At present no other type kit as accurately shows bond thickness, which is of some importance to molecular geometry. Per-

No other inexpensive kit is as accurate as this one.

haps your imagination will be taxed, since molecular shape must be inferred. After you have used this kit for a while you should become proficient in recognizing 3-dimensional relationships. We will begin with simple models and will ultimately try some complex models that will require cooperation of several sets of lab partners.

Open the kit. Put the enclosed instruction book aside. This booklet is for help in time of great need. Don't lose it.

Copper wire may be substituted for the angular fasteners in the kit. When you need extra ones, use AWG 12 Ga or any wire of ∿ 0.080 in. diameter.

Alternatively a section of tubing from the kit cut vertically into thirds yields flexible strips of the right diameter.

There are three depressions holding two types of metal parts: clusters and fasteners. In two depressions are lengths of plastic tubing. There is a shallow groove along one side that will be used for measuring tubing before it is cut.

A.2.a METAL PARTS

There are three kinds of multiarmed metal parts called clusters. Each different metal cluster is used to represent a certain type of bonding situation for an atom and not necessarily a specific element. Silver clusters have four arms and are used to indicate bond angles near 109°; brass clusters have five arms and are used to indicate hybridized bond angles of 120°; the six arms of the copper

Hybridization as presented refers only to 2nd period elements. Third and subsequent periods involve d orbitals and are very complex (see Pauling--The Nature of the Chemical Bond).

cluster represent bond angles of 90°.

Recall from Chapter 17 of CHEMS that carbon has the electron configuration

$$1s^2 2s^2 2p_x^{\ 1} 2p_y^{\ 1}$$

When a carbon atom bonds with another atom, the carbon atom "promotes" one 2s electron so that its configuration is

$$1s^2 2s^1 2p_x^{\ 1} 2p_y^{\ 1} 2p_z^{\ 1}$$

Further, once promoted, the four valence orbitals apparently "mix" to form four new identical orbitals. This process is called hybridization, and the resulting orbitals are called sp^3 hybrids from a mix of one s and 3 p orbitals.

After this discussion the clusters will be referred to by color, i.e., silver, brass or copper, or by type, such as sp^3.

Silver clusters represent sp^3 hybridization.

The designation sp^3 identifies which orbitals are "mixed," one s and three p orbitals. Therefore, sp^2 hybridization means that an s and 2 p orbitals hybridize, while the remaining p orbital is not involved. sp hybridization indicates one s and one p orbital hybridize, with the two other p orbitals not involved.

A.3 HYBRID ORBITALS

A hybrid orbital differs from ordinary s and p orbitals in several ways. First, ordinary p orbitals are symmetrical about the nucleus, but hybrid orbitals are asymmetric. (Figure A.1) Second, because they are asymmetric (and because these hybrid orbitals, just as any orbitals, are arranged in the lowest energy configuration, i.e., as far apart as possible), the actual arrangement of the orbitals in space is different for the hybrids than for

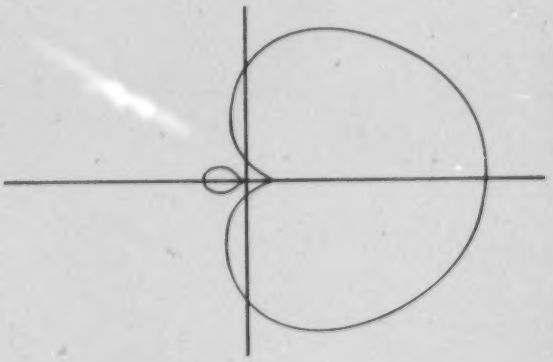


Figure A.1

A Hybrid Orbital

p orbitals. For example 3 hybrid sp^2 orbitals will be arranged at 120° to each other in a plane, while 3 p orbitals are at 90° to each other. Third, these orbitals are regions of much greater bonding tendency (as can be seen by their asymmetry) than s or p orbitals. In fact, hybridized bonds result in much greater stability for the molecule than would be predicted from p orbital bonding.

Since the four sp^3 hybrids of carbon are spaced as far apart from each other as are the arms of a tetrahedron, the angles between the bonds are 109° 28'. All sp^3 hybrid orbitals have this angle between the four bonds. This angle is also close enough to represent the bonds of oxygen in water and nitrogen in ammonia and alkyl amines. It is true, however, that most frequent use of the silver cluster will be carbon atoms that form sp^3 orbital bonds.

Water may be represented as an sp^3 hybridization of oxygen.

Electron promotion precedes all hybridization.

Under certain bonding conditions, a carbon atom forms sp^2 hybrid orbitals. The sp^2 hybrid orbitals are formed of one s and two p orbitals to make three equal hybrid orbitals. These orbitals orient themselves 120° apart and all lie in the same plane. The non-hybridized p orbital is found at 90° to the plane of the sp^2 hybrids. The brass cluster illustrates this kind

Remember that hybrid orbitals are regions of high bond probability-higher than ordinary p orbitals.

of bond situation.

A.4 SINGLE BONDS

Single covalent bonds which are linear and symmetrical about the bond axis between atoms are called "σ" or "sigma" bonds and are the covalent bonds that exist in molecules. Figure A.2 shows two ρ orbitals overlapping to form a σ (sigma) bond. C-D is the bond axis; the dotted line above F identifies the center of the covalent bond. The distance from this center to the nucleus of atom A (d_1) is the covalent bond radius of A. The distance along the bond axis from the center to the nucleus of atom B (d_2) is its covalent radius. Note that the dotted line at F is only meant to indicate the center of the σ bond and not the halfway distance along the bond axis. In some cases this bond center may coincide with the halfway distance between the atoms, but it is usually displaced toward one atom of the pair. Displacement is toward the more electronegative element.

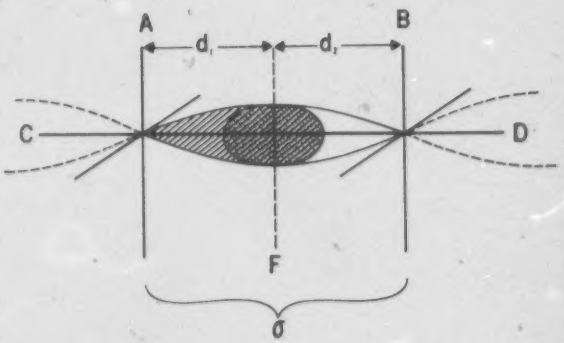


Figure A.2

It has been found that usually the bond length for the single bond A-B is, to within about 0.03 Å, equal to the average of the bond lengths A-A and B-B.

A.5 DOUBLE BONDS

When sp^2 hybridization occurs--let's say between two carbon atoms--an sp^2 hybrid from each carbon overlaps to form a σ bond, and the p orbitals that are at right angles to the plane

Some teachers have found that coloring in the orbital on the following pages with felt pens or colored pencils improves the pictures considerably.

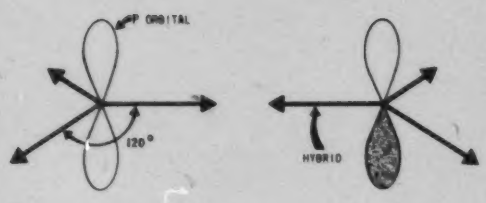


Fig. A.3
Arrows represent hybrid sp^2 orbitals

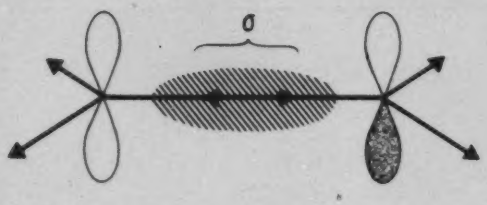


Fig. A.4
Two sp^2 hybrids form σ bond between nuclei.

At the exact same time (σ bond omitted for clarity) distortions of p orbitals leads to overlap and share of electrons, i.e., bond:

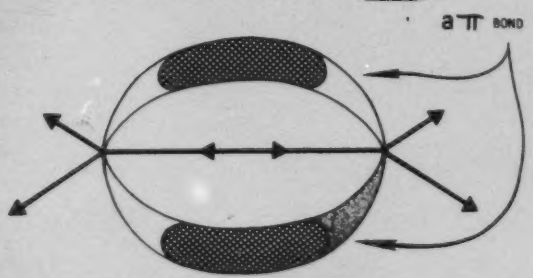


Fig. A.5
This double-armed bond is a pi (π) bond.

Since a single bar C-C represents a single covalent bond, double bars (C=C) and triple bars (C≡C) represent double and triple bonds.

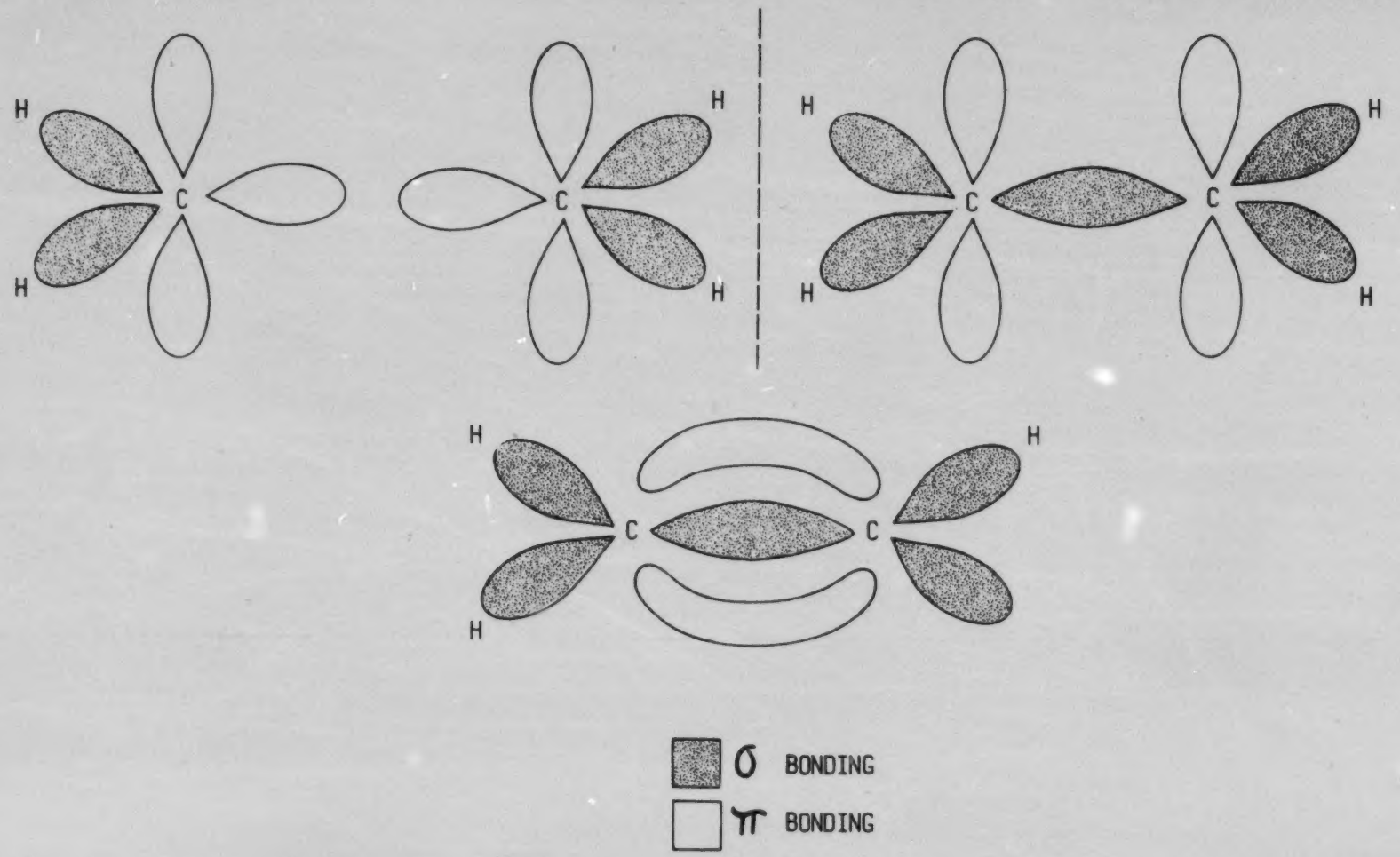
of the sp^2 hybrids can distort and form a new bond called a π (pi) bond.

(Illustrations of the orbital overlap in the pi bond formation may be seen in Figures A.3 and A.6).

Note that the π bond is joined above and below the plane of the sp^2 hybrids. This gives rigidity to this bond so the atoms are not free to turn on the axis of the σ bond. This, of course, is a double bond and is found between carbon atoms (C=C), between carbon and oxygen (C=O), and between carbon and nitrogen (C=N). Since the forces holding two nuclei together in a double bond are greater than those of the single bond the nuclei are drawn closer together, and thus the C-C covalent distance is greater than the C=C covalent distance. (Table A.1)

Atomic Covalent Radii (A°)		Single Bond Radii
C - single 0.77	H	0.30
C - double 0.67	F	0.64
C - triple 0.60	Si	1.17
	P	1.10
N - single 0.74	S	1.04
N - double 0.62	Cl	1.00
N - triple 0.55	Br	1.14
	I	1.33
O - single 0.74		
O - double 0.62		
O - triple 0.55		

Table A.1



42

ORBITAL OVERLAP IN π-BOND FORMATION IN ETHYLENE
BY OVERLAP OF P ORBITALS. AS SHOWN IN THREE STAGES

43

Figure A.6

Bond strength:

C-C	*83 kcal/mole*
C=C	*100 kcal/mole*
C≡C	*123 kcal/mole*

Morrison and Boyd, Organic Chemistry, Allyn and Bacon.

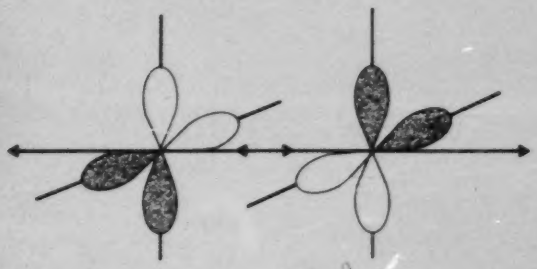


Figure A.7
p orbitals which form π bonds.

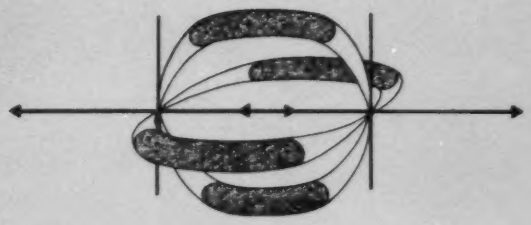


Figure A.8
π bonds formed

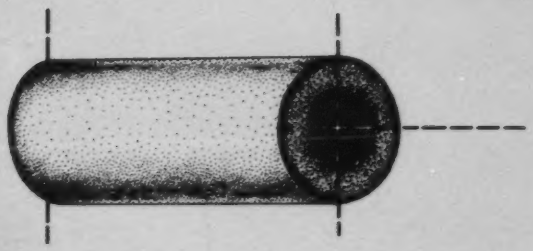


Figure A.9
Another representation of the two π bonds in a triple bond.

Although this is a double bond, it is not a double π bond but rather a strong σ and a weaker π bond with the result that the double bond is stronger than the single bond but not twice as strong.

A.6 TRIPLE BONDS

The copper-colored fastener illustrates the third kind of hybridization of carbon. In this case hybridization involves one s and one p orbital, thus giving two hybrid orbitals while retaining two ordinary p orbitals. Since the hybrids are equivalent, they assume a linear configuration. The p orbitals form at right angles to the hybrids. The two non-bonded p orbitals are found at 90° to the sp orbitals and 90° to each other. The triple bond of carbon in acetylene HC≡CH is formed by σ bond formation between sp hybrids and π bonds between the four p orbitals left.

There are two other fasteners. One is a linear pin that is used to show bonds between unlike atoms and one an angular fastener that is used in building double and triple bonds.

A.7 PLASTIC TUBING

Interatomic distances are measured in Ångstrom units, abbreviated Å. One Å is equal to 10^{-10} meters or 10^{-8} cm. We shall refer

to distances in Å. In this kit the scale is 1 in. to 1 Å.

The equivalence 1 in. = 1Å is unfortunate but dictated by the kit.

Current usage is in nanometers:

1 nanometer = 1nm = 10^{-9}m.

1nm = 10Å.

The plastic tubing is used to identify specific atoms involved in bonding. This is done through a color key found in Table A.2.

Color Coding of Atoms in Kit	
hydrogen-white	bromine-orange
carbon-black	iodine-brown
nitrogen-blue	silicon-light yellow
oxygen-red	phosphorus-violet
fluorine-light green	sulfur-dark yellow
chlorine-dark green	all metals-grey

Table A.2

The length of the plastic tubing used indicates either the covalent radius of the atom or the van der Waals radius (the non-bond direction). You will recall that the van der Waals distance is usually the non-bonded distance of nearest approach of one atom to another. Lengths of covalent radii are given in Table A.1 and lengths of van der Waals radii in Table A.3.

See CHEMS Ch. 17 for van der Waals forces.

It is very important that the students understand that the plastic tubing represents electrons involved in bonding and the cluster represents the nucleus.

van der Waals radii in Å									
N	1.50	Sb	2.20	Sc	2.00	F	1.35	I	2.15
P	1.90	O	1.40	Te	2.20	Cl	1.80		
As	2.00	S	1.85	H	1.20	Br	1.95		

Table A.3

The tubing is fastened to the metal clusters by sliding the tubing onto one of the arms of the cluster. Thus the color of the tubing on the cluster identifies the atom the cluster represents. The length of the tubing represents the covalent or van der Waals radii. The position and size of an atom are clearly visible within the framework model of the molecule.

A precise decimal inch scale is to be found in the booklet which comes with each kit.

A.8 CONSTRUCTION OF BONDS

A single-edge razor blace and a 3/4 x 4 x 4 piece of pine do nicely for cutting tubing.

A single length of black tubing 1.54 inches long is the equivalent length of two carbon covalent radii joined, i.e., one carbon-carbon covalent bond.

Bonds between like atoms are cut from tubing of one color. The length of such a bond is twice the covalent radius of the atom. For example, the C-C (carbon - carbon) bond is 1.54 Å long, since the covalent radius of C is 0.77 Å. Take a length of black tubing and as accurately as possible cut a piece to 1.54 inches (remember that the scale is 1Å = 1 inch, so 1.54 Å = 1.54 in.) by using the scale on the kit. If you are satisfied that you've cut this piece to represent 1.54 Å, mark it (with masking tape, etc.) to use as a pattern to cut all other 1.54 Å pieces of black tubing. Now cut out 10 or 15 pieces of 1.54 Å black tubing for later use. Take a 1.54 Å piece and join two silver clusters together. You have a bare C-C bond. Set this aside for now. All other bonds between like atoms can be made by cutting the proper tubing

Any covalent bond between like atoms is made by cutting tubing of the correct color to a length exactly equal to twice the covalent radius of the atom.

All preprinted tubing should be measured to see that it is the length represented or needed.

to the right length and joining the indicated clusters.

A.9 BONDS BETWEEN UNLIKE ATOMS

As an example, the C-O bond is shown. This kit provides several lengths of colored tubing already correctly printed to show C-O bonds.

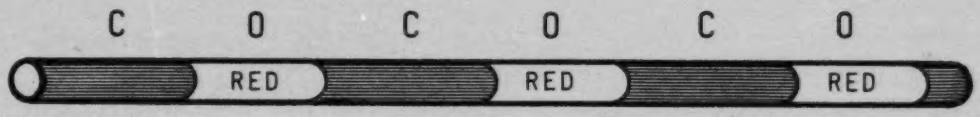


Figure A.10 CUT CUT CUT

This tubing, when cut, will yield C-O bonds of the correct length. However, these are C-O single bonds, and C-O double bonds will have to be built. The building of single bonds between atoms follows a regular procedure; only the length and color of the tubing change from one bond to the next.

A.9.a TO BUILD A C-O BOND

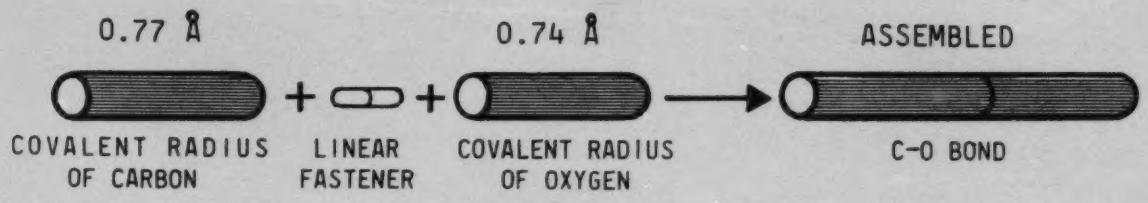


Figure A.11

Black-blue (C-N) tubing, like the C-O tubing in Figure A.10, is also included. Other bonds will

have to be constructed as you need them.

This kit provides preprinted C-H, N-H and O-H bonds. These bonds are printed as in Figure A.12. The tubing in Figure A.12 is C-H bonding; however, N-H and O-H bonds are printed (and cut)

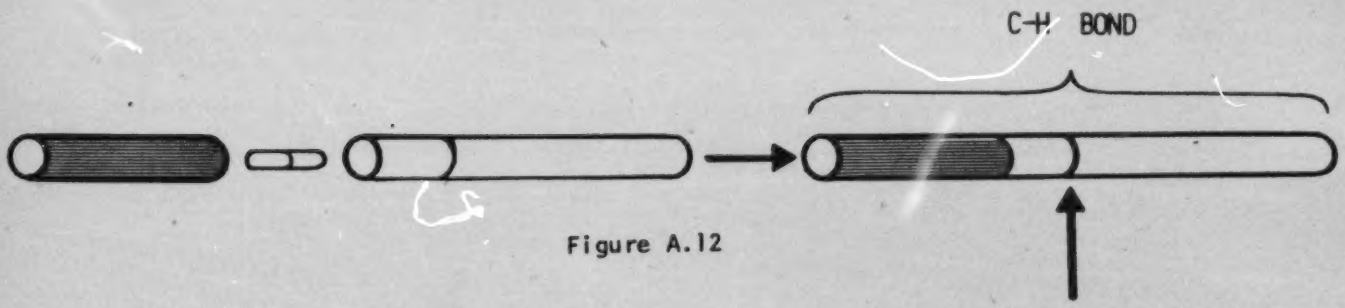


Figure A.12

An N-H bond is represented by a length of tubing. Remember to check on proper length.

in the same way.

A.10 ENOUGH TALK; LET'S BUILD MOLECULES

We will start with methane--CH_4. Take a silver(sp^3) cluster and four C-H bonds (cut as previously described). Slide the black end of each C-H bond all the way onto an arm of the cluster. Do this for all four C-H bonds.

Next make a water molecule. This model is made by attaching two O-H bonds and two sections of red tubing (cut to van der Waals radius of oxygen) to an sp^3 cluster.

One can construct the hydronium ion, H_3O^+ by removing one of the pieces of red tubing (which represents an unshared pair of electrons) and putting on an O-H bond.

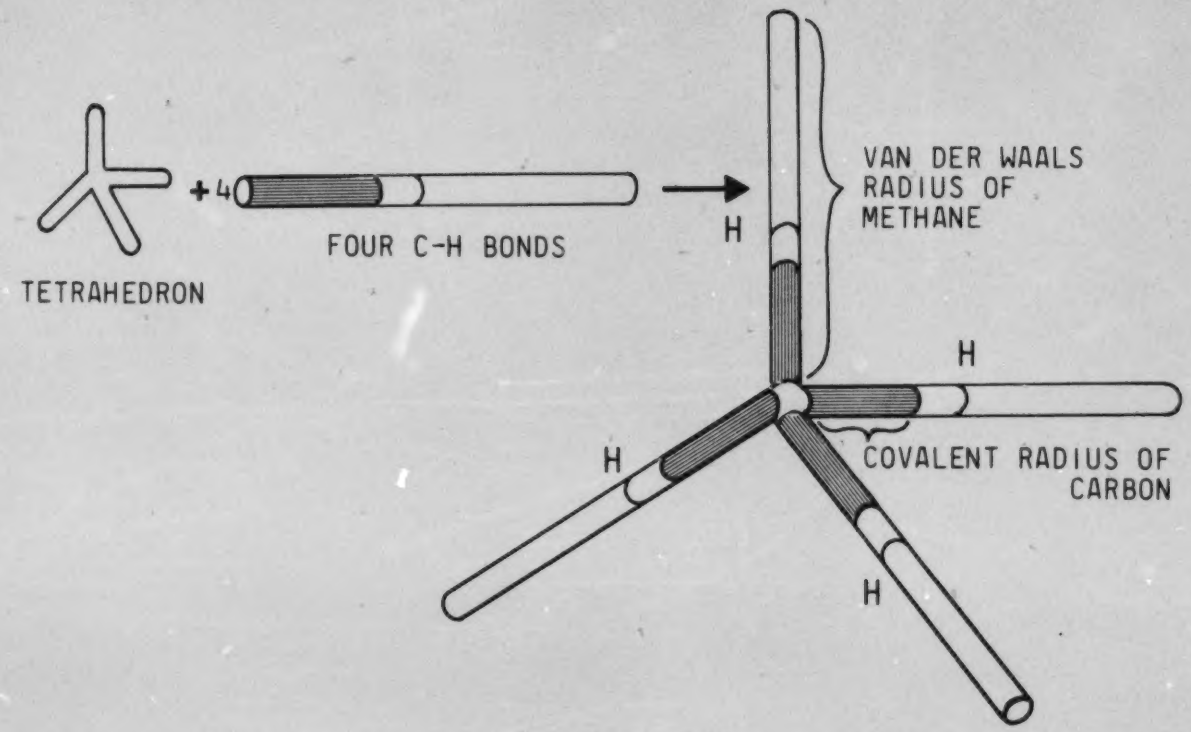


Figure A.13

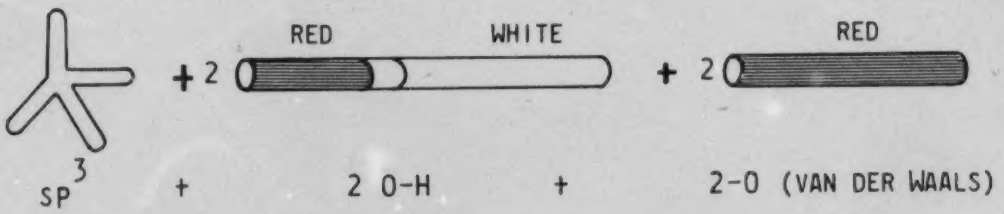


Figure A.14

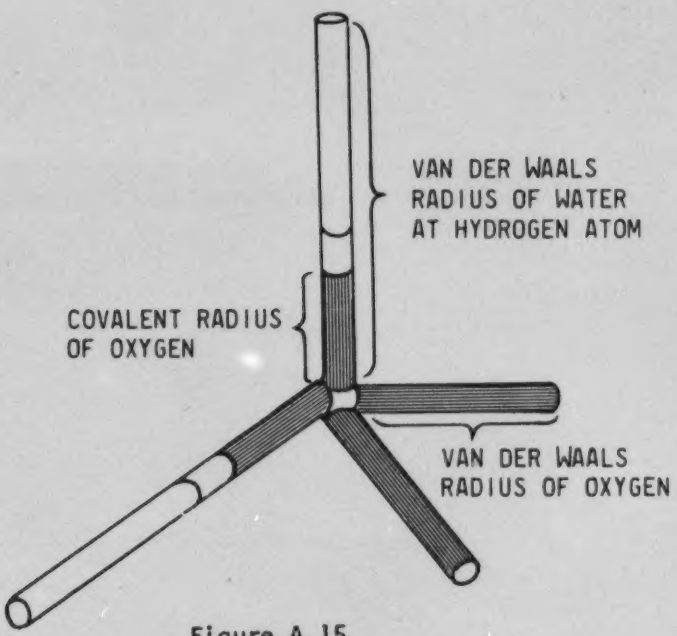
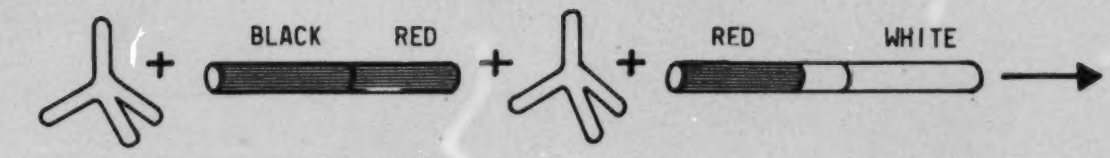


Figure A.15

Let's make a more complicated molecule, a model of methanol--CH_3OH.

To build this we will need



TETRAHEDRON FOR CARBON · C-O BOND · TETRAHEDRON FOR OXYGEN · O-H

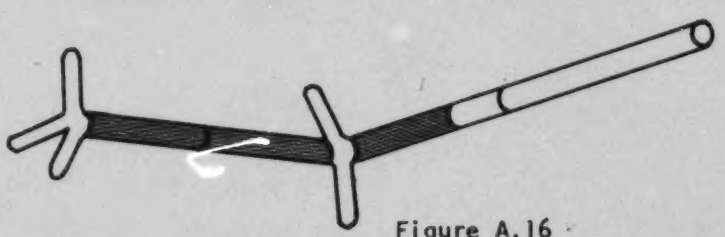


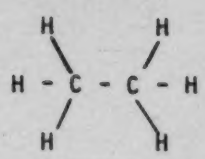
Figure A.16

Complete the model by adding C-H bonds to the carbon tetrahedron and two van der Waals lengths of oxygen (red) tubing to the oxygen tetrahedron.

The unbonded electrons occupy a space represented by the van der Waals length only when the unbonded electrons belong to a singly bonded atom.

A.11 WORDS ABOUT MOLECULES

In point of fact, you now know how to make CH_4 models and how to make models containing O-H and C-O-H groups. If you take the tetrahedra connected by black tubing which you constructed earlier and attach C-H bonds to the available arms you will have



which is CH_3CH_3 or C_2H_6 or ethane. As a matter of fact, you should now build several models of this series. Just remove a C-H bond from a tetrahedron, add a C-C bond and finish the molecule by adding C-H bonds. As you do this, you'll note that when you add a new carbon, you will also add two new C-H bonds. An entire series of carbon compounds called the alkanes is built in this way. The general formula of this group is C_nH_{2n+2} when n is any integer.

After building several of the molecules in the series CH_4, C_2H_6, C_3H_8, C_4H_{10}, C_5H_{12} there are several things you should note. One important factor is that because of the 109° bond of carbon these molecules twist around and are far from linear. Another point is that there is more than one way to put C_4H_{10} together. In fact the greater the number of carbons, the more possible shapes there are. These different possibilities are called isomers; they have the same emperical formula but are actually different compounds. A good example might be C_2H_6O, where one isomer involves C-O-C bonds (an ether) and

Alkane Series (Saturated Hydrocarbons) First Ten Members
CH_4 - Methane
C_2H_6 - Ethane
C_3H_8 - Propane
C_4H_{10} - Butane
C_5H_{12} - Pentane
C_6H_{14} - Hexane
C_7H_{16} - Heptane
C_8H_{18} - Octane
C_9H_{20} - Nonane
$C_{10}H_{22}$ - Decane

Table A.4

See Pages 36,37 for rules of nomenclature.

Bond angle for sp^3 is 109°28'.

See pages 35a,b for FUNCTIONAL GROUPS.

another C-O-H bonds (an alcohol).

You cannot twist the molecules too tightly together. Since the hydrogen atoms repel each other, they will stay as far away from one another as possible. For large molecules this can be a very complex situation. You also should note that some rotation about bonds is possible. While there is rotation about the bonds, it may be restricted by intramolecular repulsive forces. Make a model of C_2H_6. Note that in one position the hydrogens are as close as possible while in another, where one end has rotated 60°, they are as far apart as possible. In ethane molecules 3 kcal/mole are required to produce this rotation. Indicating or representing these carbon structures on paper is difficult.

In the Newman Projection for ethane, one carbon is represented by

the other by

Looking along the C-C bond we have

Also, consult any recent book on conformational chemistry.

In the structures shown so far, covalent bonds are represented by a line between atoms. Another very important type of bond is the hydrogen bond. This bond is not as strong as the covalent bond and results from hydrogen's ability to simultaneously bond to two atoms of O, N or F. This simultaneous bond has two parts. One part is covalent; the other part is electrostatic and is represented by a dashed line, O-H---O. This bond is linear, as can be seen by building some water molecules and hydrogen-bonding sev-

eral together.

What is ice? Why does ice float on water?

The hydrogen bond is electrostatic. Bond strength is dependent on angle. A linear bond, of course, is the strongest.

Water hydrogen bonds to form crystals. If the students build a small water crystal, they can see the expansion that takes place.

To build a hydrogen bond, any odd colored tubing will do. The length of a hydrogen bond is from 2.6 Å to 3.0 Å for water.

A.12 BUILDING MULTIPLE BONDS

A carbon-carbon double bond occurs in the compound ethylene, $CH_2=CH_2$ or C_2H_4. To build double bonds correctly, we must be sure we work with double-bond distances, so check Table A.1 on page 13. Next we have to use the right bonds; sp^2 clusters (brass) are used. Begin this construction by assembling the following:

A C=C bond is cut from black tubing. Cut it twice the double bond covalent radius (from Table A.1), i.e., 1.34 in. long.

2 sp^2 clusters (brass)

4 C-H bonds

3 lengths tubing 1.34 in. long

4 lengths black tubing 1.34 in. long

4 angle fasteners

The sp^2 clusters have three prongs that lie in the same plane and are 120° apart. These prongs represent sp^2 hybrid orbitals. Take one of the pieces of black tubing 1.34 inches long and attach the two sp^2 clusters to the tubing by sp^2 hybrid prongs. Now attach the four C-H bonds to the other sp^2 prongs with the black end of the tubing on the prong. (See Figure A.17 - exploded and Figure A.18 - assembled.)

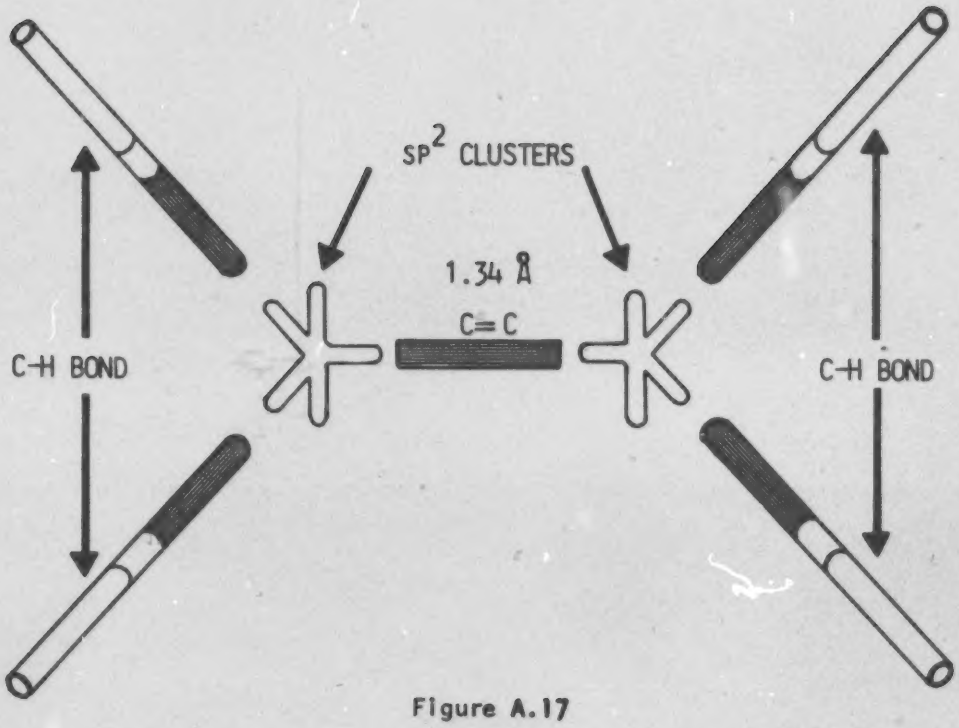


Figure A.17

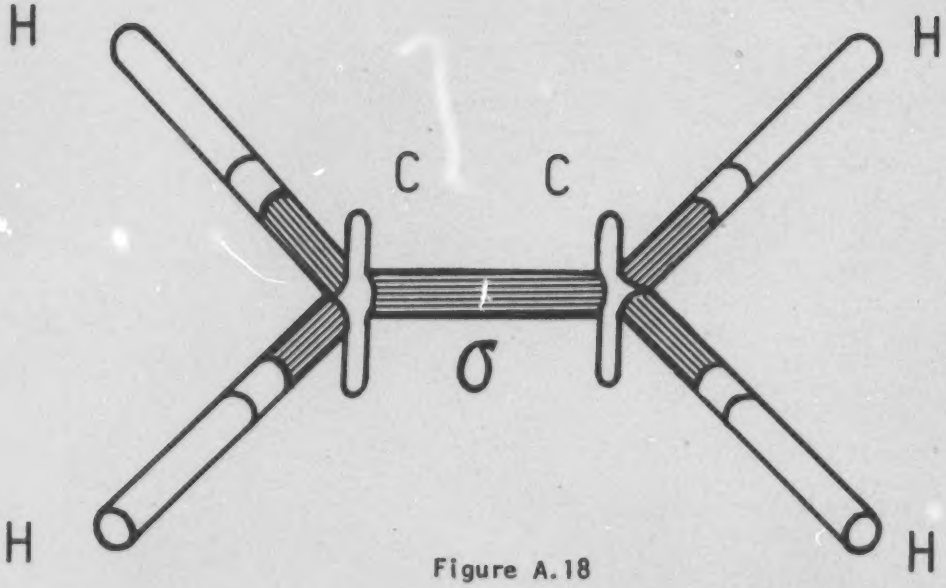


Figure A.18

Now attach the four 1.54-inch lengths of tubing to the "unbonded" prongs that represent *p* orbitals. Your model should now look like this:

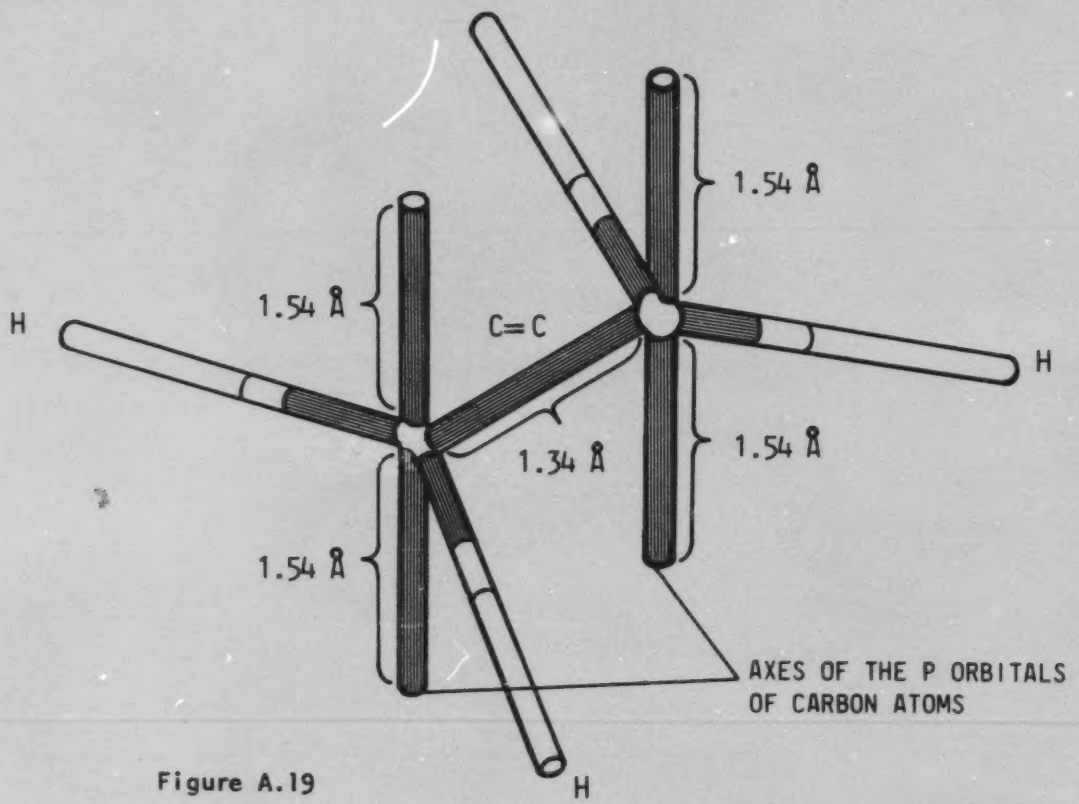


Figure A.19

We will complete the model (and the π bond) by taking two angle fasteners and sticking them into the ends of a piece of 1.34 inch-long black tubing, then joining this assembly to the open ends of two of the pieces of black tubing representing the *p* orbitals. Take the other two angle fasteners and the last piece of tubing (1.34 in. long) and attach it to the other *p* orbitals. Thus you've completed the π bond, and your model should look like this:

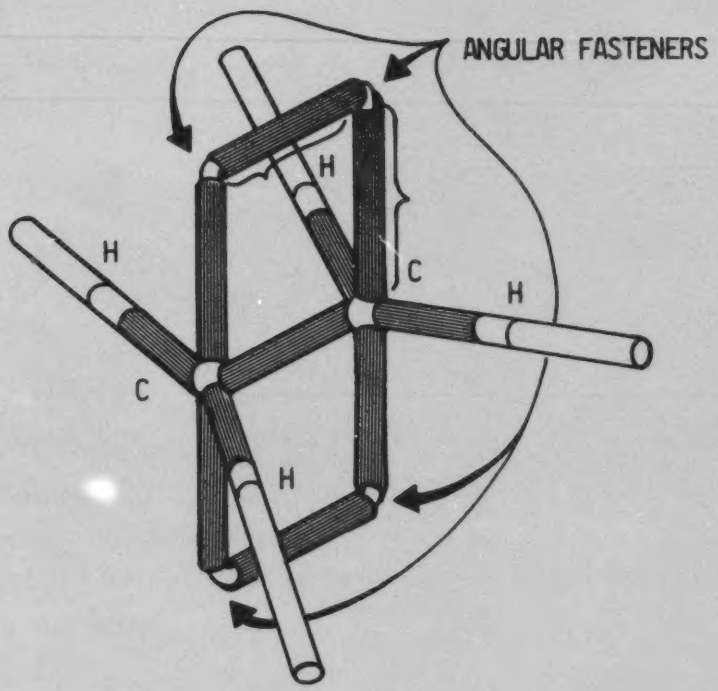


Figure A.20

The reason you used 1.54 inch-long tubing pieces on the axial *p* orbital is because the actual π bond between carbon atoms is 3.08 Å thick, and therefore this makes an accurate representation of the molecule.

When the π orbital is complete, you will not be able to rotate the ends of your ethylene molecule as you could the ends of the ethane molecule. In fact, the double bond severely restricts any rotation in the actual molecule of ethylene.. We usually assume such rotation does not happen.

Now let's make a double bond between two unlike atoms, carbon and oxygen. In constructing a π bond between a carbon atom and an atom of oxygen, the π bond thickness at the oxygen atom is represented by using tubing of twice the covalent radius of oxygen as the axial p orbital. For a C=N bond double the covalent radius of nitrogen for the axial p orbital. In both cases the axial p orbital of carbon will be 1.54 Å long.

Thickness of π bond is determined by x ray or neutron diffraction.

The simplest organic molecule that contains such a double bond is formaldehyde, structural formula $\begin{matrix} H \\ H \end{matrix}\!\!>C{=}O$. You need the following:

2 sp^2 clusters

2 lengths black tubing (carbon) 1.54 in.

2 lengths red tubing (oxygen) 1.48 in.

3 C=O bonds from kit or black 0.67 in.

+ red 0.62 in.

2 C-H bonds

2 lengths red tubing (oxygen) 0.74 in.

4 angle fasteners

If your kit contains C=O bonds, then proceed as follows; if not, first construct three bonds of this type.

Take one C=O and attach an sp^2 cluster (hybrid prongs) to each end. To the other 2 hybrid prongs on the carbon (black) side, add

29

the C-H bonds. Then add the two black 1.54-inch lengths to the p orbital prongs on the carbon cluster. Join two angle fasteners to a C=O bond and attach the whole thing to a pair of p arms, one black and one red. Remember to attach the black end of the C=O bond to the black p bond. Now add the other C=O bond and put the two 0.74-inch lengths of red tubing on the remaining hybrid prongs of the oxygen atom. Thus you've completed the formaldehyde molecule, and it should look like Figure A.21.

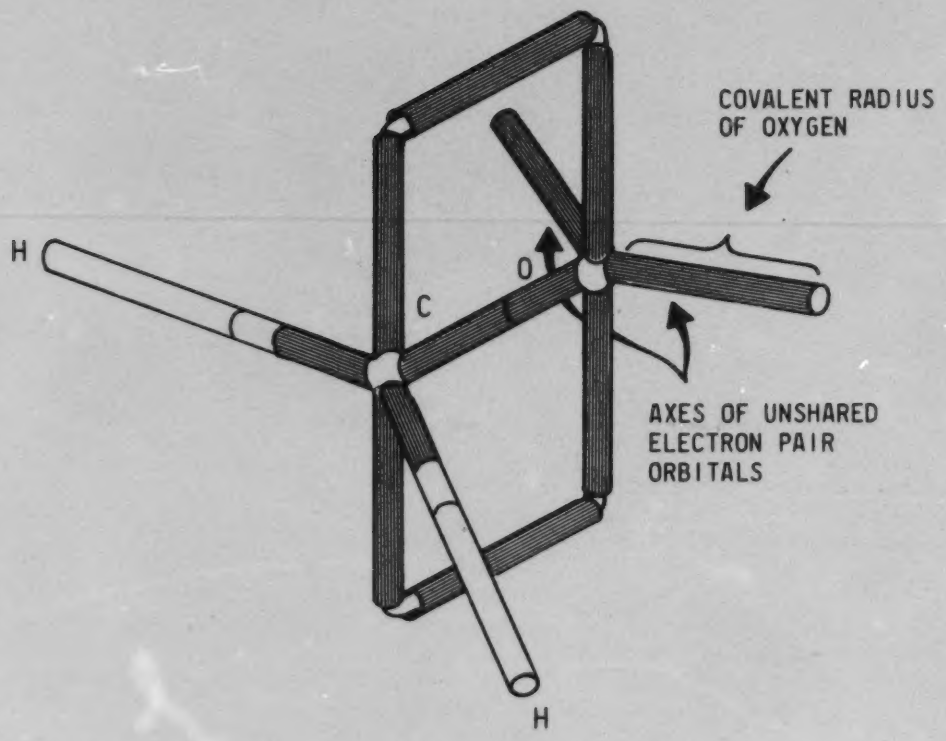


Figure A.21

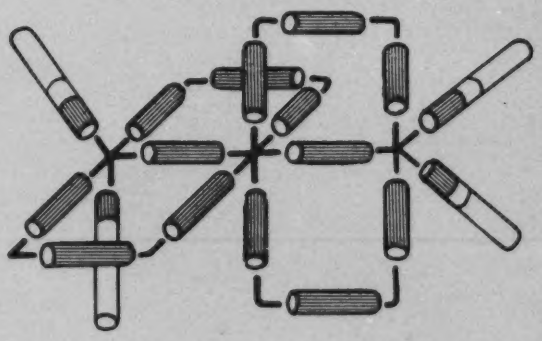
You used the van der Waals radius to model the unbonded electrons of the oxygen atoms in water. For the formaldehyde molecule you used the covalent radius because the carbon-oxygen double bond in effect "pulls" or distorts the oxygen electron cloud. Also, using 1.48-inch red tubing for the oxygen end of the π bond and 1.54-inch black for the carbon end should have indicated a certain strain on this double bond.

The above points on distortion of the electron clouds and strain should be kept in mind when building other double bonds.

Emphasize this point.

Remember that half of a covalent bond belongs to one atom, the other half to the other atom, and use tubing and color correctly. The C=N bond is built just as the C=C and C=O bonds, differing only in tubing colors.

A more difficult model is that of allene (1, 2 - propadiene). Allene has the general formula C_3H_4 and structurally is $\begin{matrix} H \\ H \end{matrix}\!\!>\!C=C=C<\!\!\begin{matrix} H \\ H \end{matrix}$. Here we have one carbon atom, the middle one, double bonded to the other two carbon atoms. This means that the middle carbon atom is π bonded to each of the other carbons. Forming two π bonds is accomplished through *sp* hybridization. In the kit this is represented by a



Allene - exploded view
Figure A.22

copper colored cluster. To build allene you will need:

1 copper cluster (sp)

2 brass clusters (sp^2)

6 C=C bonds

4 C-H bonds

8 C-C bonds

8 angle fasteners

Begin by connecting the sp cluster to an sp^2 cluster by a C=C bond (σ bond). Now build the π bond between these two. Next connect the other sp^2 cluster to the sp with a C=C bond and build the other π bond. Complete the model by attaching the C-H bonds to the sp^2 clusters.

Another kind of multiple bond is the triple bond. In this case the carbon is sp hybridized, a σ bond is formed between sp hybrids, and π bonds are formed between p orbitals. Build C_2H_2 (acetylene) by using the sp clusters and the correct bond lengths from the table.

A.13 RING STRUCTURE

One of the very interesting aspects of using this kit comes when we build ring structures. The first ring structure to be built is cyclohexane. Hexane means six carbons and cyclo means in a circle. (What would cyclopentane be?) Take six sp^3 clusters and join them

Cyclopentane = 5 carbons in a ring.

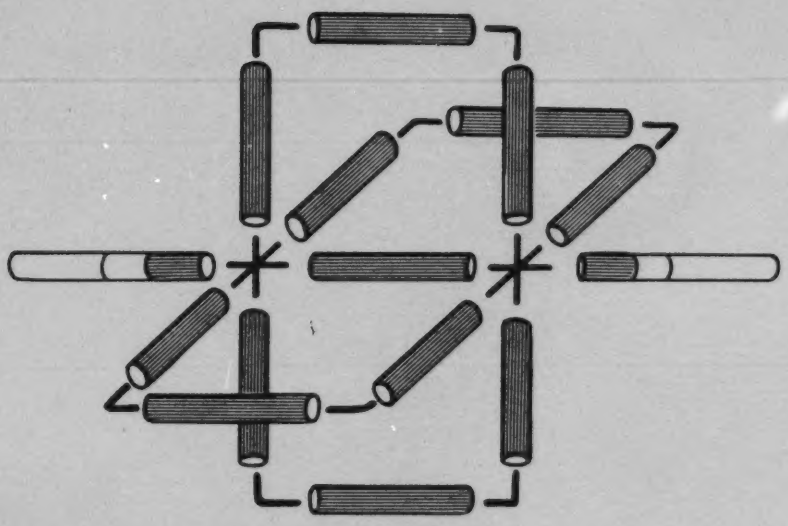


Figure A.23: Acetylene--exploded view

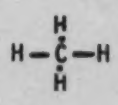
together in a ring using 1.54-inch sections of black tubing. You will note that there are two possible positions for this structure:

Figure A.24

Try making <u>cyclopentane</u>. Note that this structure also is not flat. This bent kind of conformation is called "puckering."

B. FUNCTIONAL GROUPS

As you know by now, in the language of structural diagrams the bar (or line---) represents a bond; for example



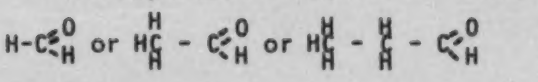
is methane, a carbon atom bonded to four hydrogen atoms. Some other useful conventions follow.

B.1 ALCOHOLS

Consider this series of compounds: methanol (CH_3OH), ethanol (CH_3CH_2OH) and propanol ($CH_3CH_2CH_2OH$). These compounds have in common the reactions of the -OH group. To discuss the reactivity of the -OH group we can usually ignore the specific carbon chain attached to it. We ignore everything other than the -OH by using the catchall letter R to stand for C_2H_5-, C_3H_7-, C_4H_9- and so on.

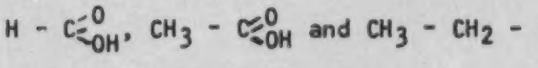
B.2 OTHER REACTIVE GROUPS

A comparable situation is found with



All of these enter into basically similar reactions, due apparently to .

Similarly



all seem to rely on $-C\overset{O}{\underset{OH}{}}$ as their source of reactivity. In other words, the molecule is reactive at the site of these different groups. The groups mentioned together with others are called functional groups. See Table B.1.

B.3 ALKYL RADICALS

In section B.1 reference was made to use of the symbol R to stand for a group of atoms attached to the reactive group. Examples of these are the alkyl radicals:

CH_3- is methyl as in

CH_3Cl methyl chloride

C_2H_5- is ethyl as in

C_2H_5OH ethyl alcohol

C_3H_7- is propyl and

C_4H_9- is butyl etc.

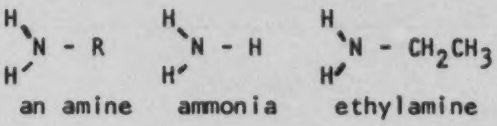
Functional Groups		
R-OH	alcohols	R - OH
R-CHO	aldehydes	R - $C\overset{O}{\underset{H}{}}$
R-COOH	carboxylic acids	R - $C\overset{O}{\underset{OH}{}}$
R-O-R	ethers	R - O - R
R-CO-R	ketones	R - $\overset{O}{\overset{\|}{C}}$ - R
R-COO-R	esters	R - $C\overset{O}{\underset{O}{}}$ - R
R-O-$\overset{H}{\underset{R}{C}}$-O-R	acetals	R - O - $\overset{H}{\underset{R}{C}}$ - O - R

Table B.1

Using the kit you should build models of all of the above structures for practice.

B.4 AMINES

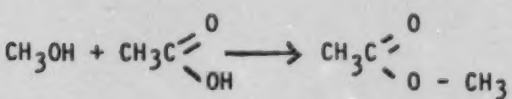
Later you will learn the importance of certain nitrogen compounds. Some of these include the amine functional group.



an amine ammonia ethylamine

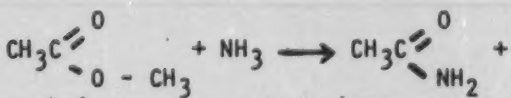
B.5 MODELS OF REACTIONS

Using the kit you can demonstrate not only molecules but also reactions. After building methyl alcohol and acetic acid, manipulate the models to show this reaction:



methyl alcohol + acetic acid ⟶ methyl acetate (an ester) + water

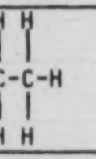
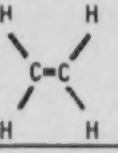
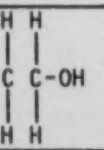
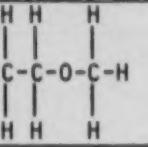
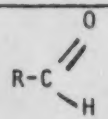
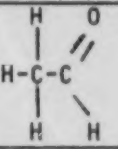
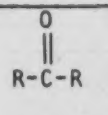
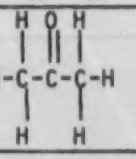
Take the methyl acetate you have just made and "react" it with ammonia.



methyl acetate + ammonia ⟶ acetamide + methanol

With the experience you've gained, you should be able to build any structure, given its structural formula.

FUNCTIONAL GROUPS - NOMENCLATURE

Functional Group	Type of Compound	IUPAC* Ending	Example	IUPAC Name	Common Name
	alkane (saturated hydrocarbon)	-ane		ethane	ethane
	alkene (olefin)	-ene		ethene	ethylene
-C≡C-	alkyene (acetylenic)	-yne	H-C≡C-H	ethyne	acetylene
R-OH	alcohol (hydroxyl)	-ol		ethanol	ethyl alcohol
R-O-R	ether	---		methoxyethane	methyl ethyl ether
	aldehyde	-al		ethanal	acetaldehyde
	ketone	-one		propanone	acetone

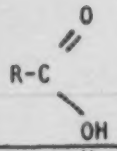
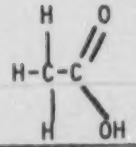
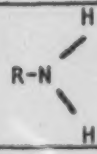
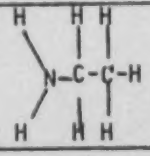
Functional Group	Type of Compound	IUPAC* Ending	Example	IUPAC Name	Common Name
	carboxylic acid	-oic		ethanoic acid	acetic acid
	amine	- - -		2-aminoethane	ethyl amine

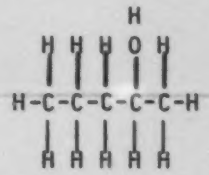
Table B.2

*International Union of Pure and Applied Chemistry

THE IUPAC SYSTEM OF NOMENCLATURE

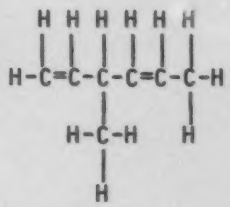
On the following page is a summary of basic IUPAC rules for naming compounds. It would pay to read through them and then go through the following examples, referring back to the rules as needed.

(1)



		Application	Rule #
a:	pent-	5 carbon atoms	#1
b:	pentan-	saturated chain (all single C-C bonds)	#2
c:	pentanol	ol-alcohol ending	#2
d:	2-pentanol		#3

(2)



a:	hexa-	6 carbon atoms	#1
b:	hexadiene	2 unsaturated bonds di=2 (two)	#2,6
c:	1,4-hexadiene	as opposed to 2,5	#3
d:	3-methyl-1,4-hexadiene	methyl group on #3 carbon	#4

IUPAC RULES

1) Using the longest continuous chain of carbon atoms (containing the functional group or groups) as the basis, name the compound as a derivative of this parent hydrocarbon. (Table A.4, p. 2)

2) Use the appropriate ending to indicate the principal functional group present. (Table B.1, p. 34, Table B.2, p. 35b)This includes the unsaturated hydrocarbon endings (-ene, -yne).

3) Number the basic carbon chain, starting at the end which will give the principal functional group the smallest possible number.

4) Name and locate by number all other substituents (other functional groups, atoms and carbon groups not part of the basic chain) attached to the longest continuous chain.

5) Substituents are listed alphabetically.

The above rules represent the most basic and should suffice in naming the majority of compounds you will run across. The following refer to more specific and less commonly occurring situations (some do occur fairly frequently though and you probably will encounter them occasionally).

6) Multiple, identical substituents are indicated by the appropriate prefix (di-, tri-, etc.) in front of its name and each is numbered.

7) Cyclic compounds are indicated by the prefix cyclo- in front of the basic name. Numbering starts with a carbon bearing a substituent and proceeds in the direction which will make the sum of the numbers of other substituents the lowest possible.

8) Complex substituents are themselves named by IUPAC rules, with this stipulation: that the carbon which is attached to the main chain is the number one carbon. To avoid ambiguity the substituent name may be placed in parentheses.

Exercises for Home, Desk and Lab (HDL's)

The first seven questions are based on the molecular models kit:

(1) What tubing color represents oxygen?
What tubing color represents carbon?
What tubing color represents nitrogen?
What tubing color represents hydrogen?

(1) red
black
blue
white

(2) Why cut tubing to 2x covalent radius? That is, why not cut covalent radii and join them?

(2) Fewer cuts, less time and models are stronger.

(3) In a C-O bond, why is part of the tubing black and the rest red in color?

(3) Part of the tubing represents the carbon atom (black) and the rest represents the oxygen atom (red).

(4) Which metal part is used in the representation of a C-C bond?

(4) Silver cluster

(5) What part of a metal cluster corresponds to the nucleus of an atom?

(5) Center of the cluster

(6) Why do sp^3 clusters have 4 legs?

(6) Each leg represents one orbital of the four sp^3 hybrids.

(7) Why does a p orbital have <u>two</u> arms to represent it, while the hybrid orbitals only have one?

(7) Ordinary orbitals are symmetrical thus two armed; hybrids are not and are represented by only one part of the orbital.

(8) Why isn't a double bond twice as strong as a single bond?

(8) A double bond is not really a 'double' anything; actually a double bond is the sum of a σ and a π bond.

(9) Why is the C=C bond distance less than that of the C-C bond?

(9) The force of multiple bonds on a nucleus is greater than that of single bonds.

(10) Are the bonds as rigid as the clusters?

(10) Not quite; almost, but not quite.

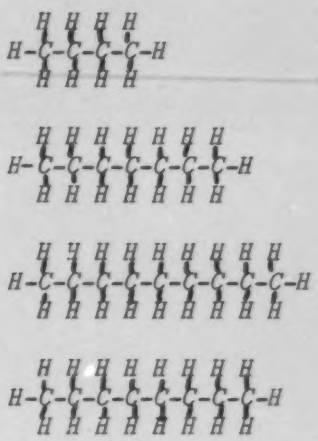
(11) For carbon, all hybrids are equivalent. If oxygen hybridizes to form sp^3 hybrids, then <u>six</u> electrons form <u>four</u> hybrids. Two of the hybrids must be "filled;" thus the four hybrids are not equivalent, and we might expect the bond angles to be different.

(11) In water the H-O-H bond angle is about 104°. Can you think of a reason why this angle is not 109° 28' if the oxygen is really sp^3 hybridized?

(12) CH_3OH

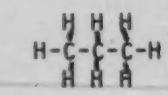
(12) Methanol and methyl alcohol are names for the same organic compound. What is its formula?

(13)



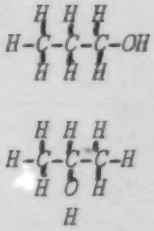
Above are the <u>simplest</u>; any isomers are satisfactory.

(13) The structural formula for propane is

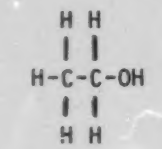


Draw structural formulas for butane, heptane, nonane and octane.

(14)

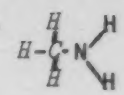


(14) The structural formula for ethanol is

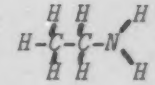


What is the structural formula for propanol?

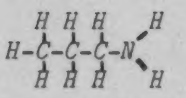
(15) a.

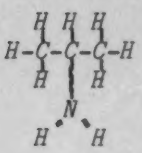


b.



(15) What are the structural formulas for (a) methyl amine, (b) ethyl amine, and (c) propyl amine?

c.  or



(16) Graphite is a material entirely composed of carbon in the sp^2 state.

a. Draw a picture of a piece of the graphite molecular structure.

b. Graphite is an isotropic material; that is, its properties in one direction through the material (electrical conductivity, heat conductivity, etc.) are very different from its properties in another direction. Explain why.

(16) a. Flat sheets of carbon covalently arranged in hexagons.

b. Conductance properties in the plane of the covalently bonded C atoms will differ (be larger) than vertical to this plane.

Because one covalent sheet of atoms can slide easily relative to the sheets above and below it, it makes a fine dry lubricating agent.

(17) Water has an unusually high molar heat capacity (amount of heat needed to raise the temperature of one mole of water one degree) and boiling point. Knowing the elements in water and the kinds of bonds they form, explain these curious properties.

(17) Both the high molar heat capacity and the high boiling point result from the high degree of hydrogen bonding. Thus in order to substantially increase the kinetic energy of liquid water, one must break many H bonds and to tear molecules free from this network (boil) one must break all H bonds.

(18) Draw all of the condensed molecular structures which fit the empirical formulas

a. C_6H_{14}

b. C_4H_8

c. C_4H_8O

(19) a. 3-Methylbutanoic acid

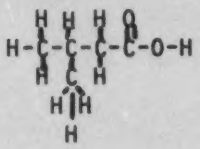
b. 2-Methylbutanal

c. 2-Propanone (acetone)

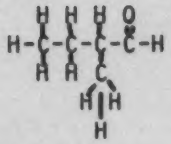
d. 2 Aminoethanoic acid (glycine or 2-aminoacetic acid)

(19) Apply the IUPAC naming system to the following organic molecules.

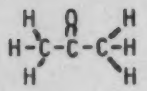
a.



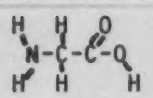
b.



c.



d.



TEXT SECTION	ROUGH TIME ESTI MATES	EXPERIMENTS	DEMONSTRATIONS	TEACHING AIDS	OTHER STUDENT ACTIVITIES	PROBLEMS	OUTSIDE READING
Chap. II Some Chemistry of Simple Carbon Compounds	↑ 1 Day ↓	CHEMS #6 for Natural Gas (optional)	Presence of CO_2 in Natural Gas		Discussion Individual library research		

Chapter II: SOME SIMPLE CARBON COMPOUNDS

One of the simplest carbon compounds is methane, CH_4. We consider methane to be uncomplicated because it contains relatively few atoms, because it contains only one type of bonding (sp^3) and because the geometry of the bonding (tetrahedral) results in a nicely symmetrical molecule. Thus, with a low molecular weight (few atoms) and little intermolecular attraction (absence of polar bonds, all atoms in the molecule have similar electronegativities, high degree of symmetry) it is not surprising that methane is gaseous under ordinary conditions. Indeed, methane boils at -161°C. (Compared with the rare gases: Argon [bp -189°, At. Wt. 40], Krypton [bp -157°, At. Wt. 84] methane with M.W. of 16 has greater intermolecular forces of attraction than do the rare gases. Compared with simple molecules composed of elements with very different electronegativities, however, it is seen that methane's intermolecular forces are weak: HF [MW 20, bp +20°C]; NH_3 [MW 17, bp -33°C].) While methane may be considered a simple molecule, the study of methane and its derivatives could lead us up many broad avenues of science including

One objective of this chapter is to drive home the thought that organic chemistry impinges on our everyday lives in a very substantial way. Even the simplest organic compound, methane, demonstrates this. The lesson could be more elaborate by using examples such as commercial polymers and the natural materials they have replaced with considerable economic turmoil; medicines and increased longevity (the population problem caused by improved drugs while alleviated by better methods of contraception); nerve gases and other organic chemical warfare agents and the moral questions such as why is it allowable to kill with bombs but not with gas, and on....

chemical technology, biology and even economics, politics and other social sciences.

A. ORIGINS OF METHANE

A.1 PRIMORDIAL METHANE

The Russian biochemist A. I. Oparin in 1936 postulated that the primordial atmosphere of the earth was mainly methane, ammonia, hydrogen and water vapor. Interestingly enough, methane, ammonia and hydrogen are probably present now in the atmospheres of Jupiter and Saturn. There is a reasonable basis for expecting methane to be a primordial gas. In the molten surface of the primordial earth the temperature was sufficiently high so that the carbon present was in the form of metal carbides. Direct chemical union of many metals with carbon to form metal carbides occurs at 2200°C and above. Some metal carbides, such as aluminum carbide, Al_4C_3, and beryllium carbide, Be_2C, react with water to yield methane.

$Al_4C_3 + 12\ H_2O \rightarrow 4Al(OH)_3 + 3CH_4$.

Thus, as the earth cooled and water vapor condensed on the surface, conditions became suitable for the production of methane. Calcium carbide yields acetylene with water:

$CaC_2 + 2H_2O \rightleftarrows Ca(OH)_2 + HC{\equiv}CH$

Acetylene may react with hydrogen to produce

ethylene or may break apart, depending on temperature, to yield more methane.

$H-C\equiv C-H + H_2 \rightarrow H_2C=CH_2$

or

$H-C\equiv C-H + 3H_2 \rightarrow 2CH_4$

The primordial methane is no longer in our atmosphere. Hurray! Where is it?

The carbon is now in the fossil fuels oil and coal, in atmospheric CO_2 and in the carbonate of sedimentary rocks.

A.2 METHANE OF BIOLOGICAL ORIGIN

In a wide variety of oxygen-free environments a certain group of bacteria are capable of living and growing on energy derived from the reduction of carbon dioxide by hydrogen to form methane and water. The detailed mechanism of how these anaerobic bacteria operate is not now known but is the subject of active research. These bacteria thrive in the sediment at the bottom of bodies of still water. The methane produced under these conditions is called marsh gas. In the rumen of cattle methane is produced by the same reaction. Bloated cows belch methane!

Evidence has been published that Methanosarcina barkerii and Methanobacterium formicum, two anaerobic soil bacteria, oxidize CO to CO_2 in the absence of H_2 or reduce CO directly to methane in the presence of H_2. (See Science Vol. 172, p. 1229 (1971).)

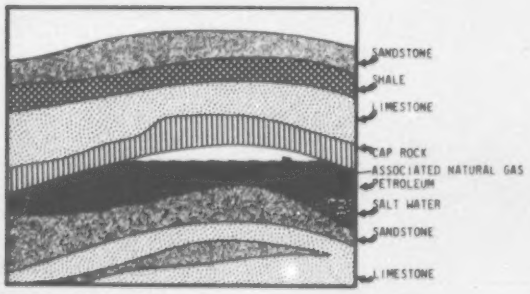
One of the purposes of sewage treatment is the removal of organic matter from water. Many sewage plants utilize anaerobic bacteria which convert the organic material to CO_2 and methane. The overall process may be represented by the equation:

$C_6H_{12}O_6 + aH_2O \rightarrow bCO_2 + cCH_4$

In this case not only is organic matter removed from the water but in addition the methane is collected as a valuable by-product.

Methane is often found in coal mines. In this case it is known as fire-damp and has been the cause of destructive fires and explosions. Some coal mines have had to be abandoned because of the fire-damp. It is not certain that bacteria are responsible for fire-damp but they may be.

A.3 METHANE FROM PETROLEUM



Petroleum is a mixture of hydrocarbons which has been trapped in certain geological formations. The proportions of the different possible hydrocarbons differs from one oil field to another or even from one well to the next within a single field. The most valuable material in crude petroleum is the mixture of hydrocarbons used for gasoline. These consist mainly of n-hexane and its isomers, (C_6H_{14}), n-heptane and its isomers, (C_7H_{16}), and n-octane and its isomers, (C_8H_{18}). Simple distillation (refining) of crude oil yields a mixture with a boiling range of about 40° to 225°, which may be used as motor fuel. The bulk of most crude oils does not distill in this range and consists of hydrocarbons with molecular weights too high to be

useful as motor fuels. In order to increase the yield of gasoline the petroleum industry has developed methods for breaking down higher molecular weight hydrocarbons into hydrocarbons suitable for motor fuel. This process is known as catalytic "cracking." Cracking may be caused by high temperature (600-800°C, thermal cracking). In both cases hydrocarbons are broken down into smaller fragments including, among other things, methane (CH_4), hydrogen, ethane (H_3C-CH_3), ethylene ($H_2C=CH_2$), propylene ($CH_3-CH=CH_2$) etc. Petroleum refineries with catalytic crackers are usually combined with petrochemical plants which use these by-products as starting materials. For instance, hydrogen is combined with nitrogen to produce ammonia.

$N_2 + 3H_2 \rightleftarrows 2NH_3$

Ethylene and propylene are polymerized to produce polyethylene and polypropylene, respectively. Methane utilization will be discussed later.

Crude petroleum contains methane and the other low molecular weight hydrocarbons which are gases at ordinary temperatures. In addition to these dissolved gases, pockets of natural gas are often found trapped in the same rock formation with petroleum and in direct contact with the crude oil. This gas is called associated

One could speculate on whether or not the outlawing of flaring forced the oil industry to develop the now highly profitable petrochemical industry.

natural gas. These gases are removed from the crude oil. In the past they were simply burned at the oil field in a spectacular flame. This practice, known as flaring, has been outlawed in most of the United States but is still done in the Middle East and in Venezuela.

A.3.a PETROLEUM DERIVATIVE PRODUCTS

These volatile hydrocarbons are now regarded as a valuable resource. The methane has many uses, which we will discuss. The others are used in several ways. For instance, by compressing them until they liquefy a useful product, liquefied petroleum gas (LPG), is obtained. Propane ($CH_3CH_2CH_3$) and butane ($CH_3CH_2CH_2CH_3$) can be treated with a dehydrogenation catalyst and converted to olefins.

$$CH_3CH_2CH_3 \xrightarrow{\text{Cat. + high temp.}} H_2 + CH_3\text{-}\overset{H}{C}\text{=}CH_2$$

Propene

$$CH_3CH_2CH_2CH_3 \xrightarrow[\text{temp.}]{\text{Cat.high}} H_2 + CH_3\text{-}CH_2\text{-}CH\text{=}CH_2$$

1 - butene

$$CH_3\text{-}CH_2\text{-}CH\text{=}CH_2 \xrightarrow{\text{further reaction}} H_2 + H_2C\text{=}\overset{H}{\overset{|}{C}}\text{-}\overset{H}{\overset{|}{C}}\text{=}CH_2$$

1,3-butadiene

Butadiene is polymerized to butyl rubber, a synthetic substitute for natural rubber.

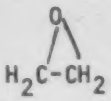
By oxidation of the various olefins avail-

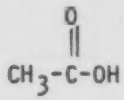
able from the dehydrogenation process a vast variety of oxygen-containing derivatives are produced. Included among the simplest of these are the following:

Ethyl alcohol, isopropyl alcohol, acetone and propylene glycol are common industrial solvents. Ethylene oxide is a sterilant as well as a chemical intermediate. Ethylene glycol is permanent antifreeze.

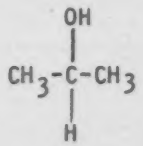
Ethyl alcohol CH_3CH_2-OH

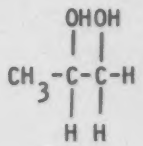
Acetaldehyde

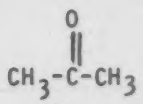
Ethylene Oxide 

Acetic acid 

Ethylene glycol $HO-CH_2-CH_2-OH$

Isopropyl alcohol 

Propylene glycol 

Acetone 

How many of these products are you familiar with? What are they used for?

A petroleum refinery and petrochemical operation is necessarily a huge industrial plant with many interconnected processes. These plants are designed so that the end products can be varied as the demand for them shifts. The only

thing that is fixed is that the starting material is always crude oil. If the supply of crude oil should fail the entire operation would screech to a halt. The technical problems of stopping and restarting such a complicated operation are so formidable that great pains are taken to insure a continuous supply of crude oil. On more than a few occasions this has involved political deals and commitments at the international level. Another problem that may develop results from the fluctuations in the demand for the main product, gasoline. Sometimes the demand for many of the products may be high while the demand for gasoline is low. It may be economically sound under these conditions to keep operating while attempting to sell more gas. Any "extra" gas that is sold must necessarily come out of the sales of some other oil company. In retaliation price cuts are implemented and prices wars develop. By restricting the price cuts to certain geographic areas most of the available gasoline is being sold at normal prices and only the "extra" gasoline is being discounted. Eventually, in the geographic area of the price war, equilibrium is reestablished but at a lower price. Each company maintains

its share of the market and there is no advantage to continue the war. The price rises to the original level, everyone with foresight has a tank full of cheap gas and the company with the surplus gasoline looks for a new locality in which to cut prices and attempt to increase its share of the market.*

A.4 NATURAL GAS

The bulk of the methane now used comes from dry natural gas. This is natural gas trapped in rock formations that is not in contact with crude oil. The adjective "dry" distinguishes such gas from the volatile distillation product of crude oil (also mainly methane) which is known as "wet natural gas." Dry natural gas may be 60 to 90% methane with nitrogen, carbon dioxide, water vapor, helium and other gases present. If the gas contains smelly sulfur compounds or a high proportion of CO_2 it is called sour gas. These contaminants are removed (in part) before sour gas is used. The recovered sulfur constitutes an important source of this element. The recovery of the helium is economically feasible at a concentration as low as 0.3%. Most of the world's supply of helium is obtained from natural gas found in the United

*There are, of course, other reasons for gas wars as well, such as local competition.

Chem Study Exp't #6 on the determination of the molecular weight of a gas may be used to determine the apparent molecular weight of natural gas. Be careful - mixtures of natural gas and air are explosive! The presence of CO_2 in natural gas may be demonstrated by bubbling natural gas through saturated calcium hydroxide solution.
$Ca(OH)_{2aq} + CO_2 \rightarrow CaCO_3\downarrow + H_2O$
Don't let much gas escape. If the ppt. doesn't appear in a few minutes, stop. The amount of CO_2 in natural gas varies.

States. The U. S. Government has carried out a helium conservation program and maintained a helium research laboratory for many years. There is much fear now that these activities will be stopped for budgetary reasons. Once lost to the atmosphere, helium could not be recovered economically.

The main use of natural gas is for energy. About one-third of the energy required in the U. S. comes from natural gas. The collection of the gas in the Southwest and in Alberta, Canada, uses about 60,000 miles of pipe lines. The high-pressure long-distance transmission of the gas uses 200,000 miles of pipe lines and the low-pressure distribution system accounts for 435,000 miles of pipe line. It is not surprising that the natural gas industry started and waxed rich in the United States. The political stability uniformly encompassing an enormous area of land that included both the source and the consumer made the investment an attractive one. In Europe progress has been slower, but the need for energy is now breaking across political borders. Perhaps this suggests that the need to solve the problems of survival may overcome the trivial obstacles as the survival problems become more acute. One highly imaginative approach

to the problem of supplying Europe's energy needs was implemented in 1964. This involved building a liquefaction plant in Algeria capable of liquefying methane (at -161°C., one atmosphere pressure), several highly insulated tanker ships and a host of terminal facilities and insulated storage tanks. Approximately $200,000,000 was spent and the governments of Algeria, France and England were heavily involved as were at least half a dozen international corporations. All the technical problems were solved and liquefied methane was shipped by boat from Africa to Western Europe. Just as the process proved itself, it was shown that a natural gas field discovered in Holland was large enough (third largest in the world) to supply much of Europe's needs. Following this discovery, natural gas was discovered under the North Sea. Western Europe's energy needs appear to be satisfied for now.

See American Scientist, Vol. 53, No. 2 and No. 3, 1965 for an account of these events.

Meanwhile, back in the U. S., the picture is not quite so rosy. Demand for natural gas is soaring, reserves are falling and shortages developing. The gas producers claim that the Federal Power Commission, which regulates the prices on interstate shipments of gas, has set prices so low it doesn't pay to drill new wells.

Some critics of the industry claim that the producers may be blackmailing consumers for higher prices by hiding reserves. Prices will probably rise some, not enough to satisfy the producers, too much to satisfy the consumers and no one will be happy. (See the Wall Street Journal, April 12, 1971.)

B. USES OF METHANE

B.1 ENERGY SOURCE

The main use of methane is burning it in air (combustion). This reaction releases energy contained in the chemical bonds (potential energy) and gives off heat.

$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O + \text{Heat}$

The heat produced is not only used to warm buildings, fry eggs and heat bath water. A significant amount of electricity is produced from methane by using the heat of this reaction to vaporize water to drive turbine generators. Since the only chemical products (under ideal conditions) are carbon dioxide and water, this is an exceedingly clean fuel.

The combustion of pure methane under standard conditions yields 212.8 kilocalories/mole (13.3 kilocalories/gram). Because of the impurities (N_2 and CO_2) in natural gas, it usually has a slightly lower standard heat of combustion-

approximately 12.5 kilocalories/gram.

Compared to other fuels natural gas is very efficient.

Crude Oil	10.5 Kcal/gram
Gasoline	11.5 Kcal/gram
Kerosene	11.0 Kcal/gram
Coal	6.8 Kcal/gram
Charcoal	8.1 Kcal/gram
Wood	
Beech	4.2 Kcal/gram
Oak	4.0 Kcal/gram
Pine	4.4 Kcal/gram

There are certain advantages (such as cost) in using less efficient fuels, but there are also significant disadvantages as well.

Some class discussion or individual library research projects may be desirable on the comparison of fuels. Topics could include:

Strip Mining
Air Pollution from by-products such as fly ash, mercury, CO, sulfur compounds, oxides of nitrogen
Water Pollution from scrubbing water used to minimize air pollution
Historically the destruction of enormous forests to make charcoal for iron reduction
Energy needs for the future

B.2 CHEMICAL INTERMEDIATES

Methane is the starting material for many important products. The simplest of these is plain old carbon.

B.2.a CARBON BLACK

Man discovered at a relatively early point that decoration was desirable and enhanced life. Pigments used for this purpose were developed from the materials available such as various plant products and minerals. The first material used for blackening was probably charcoal ground to a fine powder. By the time of the Greek civilization it was understood that a superior black pigment was obtained from the soot from burning pitch. This was the first carbon black. Fine particles of carbon, approximately 100 to 4,000 Å in diameter, are still

The formation of CO_2 and H_2O from methane in a bunsen burner flame can be demonstrated using limewater to test for CO_2. By limiting the amount of air to the burner CO should form (Perhaps Year I CO test can confirm its presence) as well as carbon black (soot).

regarded highly - in the U. S. alone, more than two billion pounds are produced annually. And a large share is produced from methane. Several processes are in use but they basically use the same chemical reaction, the thermal decomposition of methane.

$$CH_4 + \text{heat} \rightarrow \underset{\text{black}}{\underset{\text{carbon}}{C}} + 2H_2$$

In one process methane is burned in a large chamber without enough oxygen to burn all the methane in the chamber. The heat generated by the methane that is burned causes the remaining methane to thermally decompose. In the second process an oven is heated by burning methane and then methane is admitted to the oven without oxygen and it undergoes the thermal decomposition reaction. This cycle is repeated continuously.

Most of the carbon black produced is used to strengthen rubber. Automobile and truck tires utilize as much as 90% of the carbon black produced.

Black printing ink is made from carbon black and hydrocarbons such as mineral oil. The necessity for removing the carbon black from old newspapers before they can be reused as newsprint is one of the drawbacks to extensive re-

cycling of newsprint. One newspaper, the Louisville Courier Journal, has made an effort to recycle old newspapers. They find that the cost of collecting and deinking makes the recycled newsprint cost $3 more per ton than newsprint made directly from trees. The avoidance of solid waste disposal problems as well as the saving of trees might overweigh the otherwise unfavorable economics of the situation.

Does your local newspaper use any recycled newsprint? One corporation, Omark, which manufactures chain saws and other equipment used in the pulp wood industry, prints its reports to stockholders on recycled paper. This is a striking example of corporate morality.

B.2.b METHANOL

Methanol (methyl alcohol), the simplest alcohol, CH_3OH, used to be made industrially by decomposing wood. Hence, the common name for this substance is wood alcohol. Now most methanol is made indirectly from methane. The process involves several steps. We will discuss the steps separately. The last step is the reduction of carbon monoxide with hydrogen:

$$CO + 2H_2 \xrightarrow[250\text{-}400^\circ C]{200\text{-}300 \text{ atm. pressure}} CH_3OH$$

Notice that the mole ratio of 2 moles hydrogen to one mole carbon monoxide balances the

reaction and there are no by-products formed. The real success of this process depends on a means of supplying the starting material in this ratio. As a first attempt the reactions of methane and water at high temperature were used:

1) $CH_4 + H_2O \rightarrow 3H_2 + CO$

2) $CH_4 + 2H_2O \rightarrow 4H_2 + CO_2$

These reactions, while useful, yield too much hydrogen for the amount of carbon monoxide produced and, in addition, afford the by-product CO_2. These difficulties were overcome by the use of a third reaction:

3) $CO_2 + H_2 \rightarrow CO + H_2O$

The process is now carried out according to the following equation:

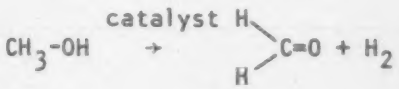
$$3CH_4 + CO_2 + 2H_2O \xrightarrow[\text{pressure}]{\text{high temp. \&}} 4CO + 8H_2$$

Note that the mole ratio of the two products fits the requirements of the methanol reaction perfectly!

The chemical plants used for methanol production are often built so that by changing the starting materials from hydrogen and carbon monoxide to hydrogen and nitrogen, ammonia may be produced instead of methanol. Nearly the same conditions are employed for both syntheses and this added versatility makes the investment

more attractive.

Methanol production in the U. S.is now at an annual rate of nearly 500,000,000 gallons. About half of it is converted to formaldehyde by several processes:



Formaldehyde is used in making plastics, resins (for plywood for example) and as an intermediate for making still other intermediates. The methanol not used for formaldehyde production either is used as an industrial solvent, an intermediate in the production of plastics, a cheap antifreeze, fuel or in the synthesis of other intermediates.

B.2.c OTHER CHEMICALS

Methane is used as starting material for so many other chemicals we could not attempt to give an exhaustive discussion of them all. The following reactions should serve to demonstrate the scope of products derived from this "simplest" organic compound.

You should be able to find at least several uses for each of the products given.

$$2CH_4 \xrightarrow{1250^\circ} H\text{-}C{\equiv}C\text{-}H + 3H_2$$

acetylene

$$CH_4 + 4S \rightarrow \underset{\text{carbon disulfide}}{CS_2} + \underset{\text{hydrogen sulfide}}{2H_2S}$$

$$CH_4 + Cl_2 \rightarrow \underset{\text{methylchloride}}{CH_3Cl} + HCl$$

$$CH_4 + 2Cl_2 \rightarrow \underset{\text{methylene chloride}}{CH_2Cl_2} + 2HCl$$

$$CH_4 + 3Cl_2 \rightarrow CHCl_3 + 3HCl$$

chloroform

$$CH_4 + 4Cl_2 \rightarrow CCl_4 + 4HCl$$

carbontetrachloride

$$CH_4 + NH_3 + 1.5\ O_2 \rightarrow \underset{\text{hydrogen cyanide}}{HCN} + 3H_2O$$

TEXT SECTION	ROUGH TIME ESTI-MATES	EXPERIMENTS	DEMONSTRATIONS	TEACHING AIDS	OTHER STUDENT ACTIVITIES	PROBLEMS	OUTSIDE READING
Chap III Polymers or stringing mono-mers together A.1 Building models of mono-mers & polymers	1 Day			Model building		1-5	
	½ Day	A.1.a Prep-aration of a polyamide--nylon				6-10	
A.2 Proteins, nucleic acids, carbohydrates	1 Day						
	1 Day	A.2.a Poly-mer size				11-18	
A.3 A Closer look at size	½ Day						
	1 Day	A.3.aPoly-mer size Quantita-tive					

Chapter III: POLYMERS OR STRINGING MONOMERS TOGETHER

A.1 BUILDING MODELS OF MONOMERS AND POLYMERS

See:

Scientific American, Sept. 1957 or Reprint No. 314 "Giant Molecules" Herman F. Mark

Scientific American, Sept. 1957 or Reprint No. 7 "Proteins" Paul Doty

The molecules which were discussed in Chapter I had molecular weights ranging from 16 for methane to 142 for decane. However, some molecules have molecular weights as high as several billion. (See Table A.1) In living systems such molecules are formed by the joining together of many small molecules in a repeating, chain-like structure of great length. These are called polymers.

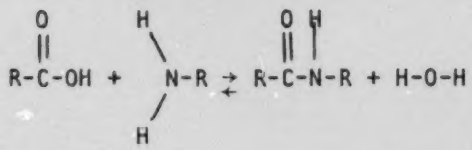
POLYMER SIZES

Name	Type	Molecular Weight
Insulin	protein	5.8×10^3
hemoglobin	protein	6.8×10^4
β-galactosidase	protein	5.3×10^5
myosin	protein	6.2×10^5
E. coli chromosome	DNA	2.8×10^9
transfer RNA	RNA	2.5×10^4

Table A.1

Construct two models of the amino acid glycine, $H_2N\text{-}CH_2\text{-}C(=O)\text{-}OH$. Glycine is bifunctional because it has two functional groups, amine and carboxyl. Carboxyl groups can react with amines

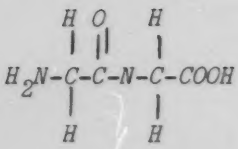
to form amides.



carboxylic acid — amine — amide — water

an amide bond

Join the two glycine models by forming an amide bond between them. The product is glycylglycine. Does the resulting amide model still have a free carboxyl and amine group?



Yes.

Join your amide model to your neighbor's model. How many amide bonds are represented in the resulting model? Does this model have any free amine or carboxyl groups? How many original glycine models are now involved in this polyamide model?

Three amide bonds.

Yes.

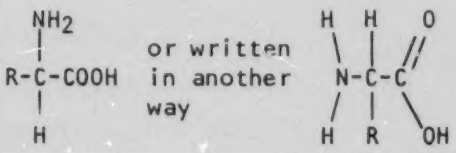
Four glycine models.

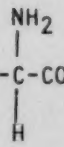
Even though the amine and carboxyl groups of the original glycine models are part of amide bonds, the number of glycine models used to make the polyamide model can still be counted.

The process of combining small molecules in a repeating fashion, as you did with glycine models, is known as polymerization. The small molecules are monomers. The resulting large molecule is a polymer. Each repeating unit in

the polymer is called a residue.

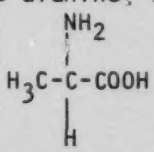
Glycine is a member of a series of compounds, the α amino acids which have the general structure:





For glycine the R group represents -H;

for the amino acid alanine, R- represents $-CH_3$:



There are 20 amino acids commonly found in living material, each differing only in the composition of the R group. Could you have a polyamide composed of different monomer units? The amide bond is also known as the peptide bond. The terms are synonymous. Therefore, a polyamide is also known as a polypeptide. Proteins are polypeptides (polyamides) having amino acids as the monomers.

Yes, composed of amino acids; the atoms involved in forming the amide bond are identical in all 20 amino acids.

It is also possible to have polyamides which do not have amino acids as monomers. If a diamine forms amide bonds with a dicarboxylic acid, then a polyamide will form. Nylon is an example.

Formation Of Nylon

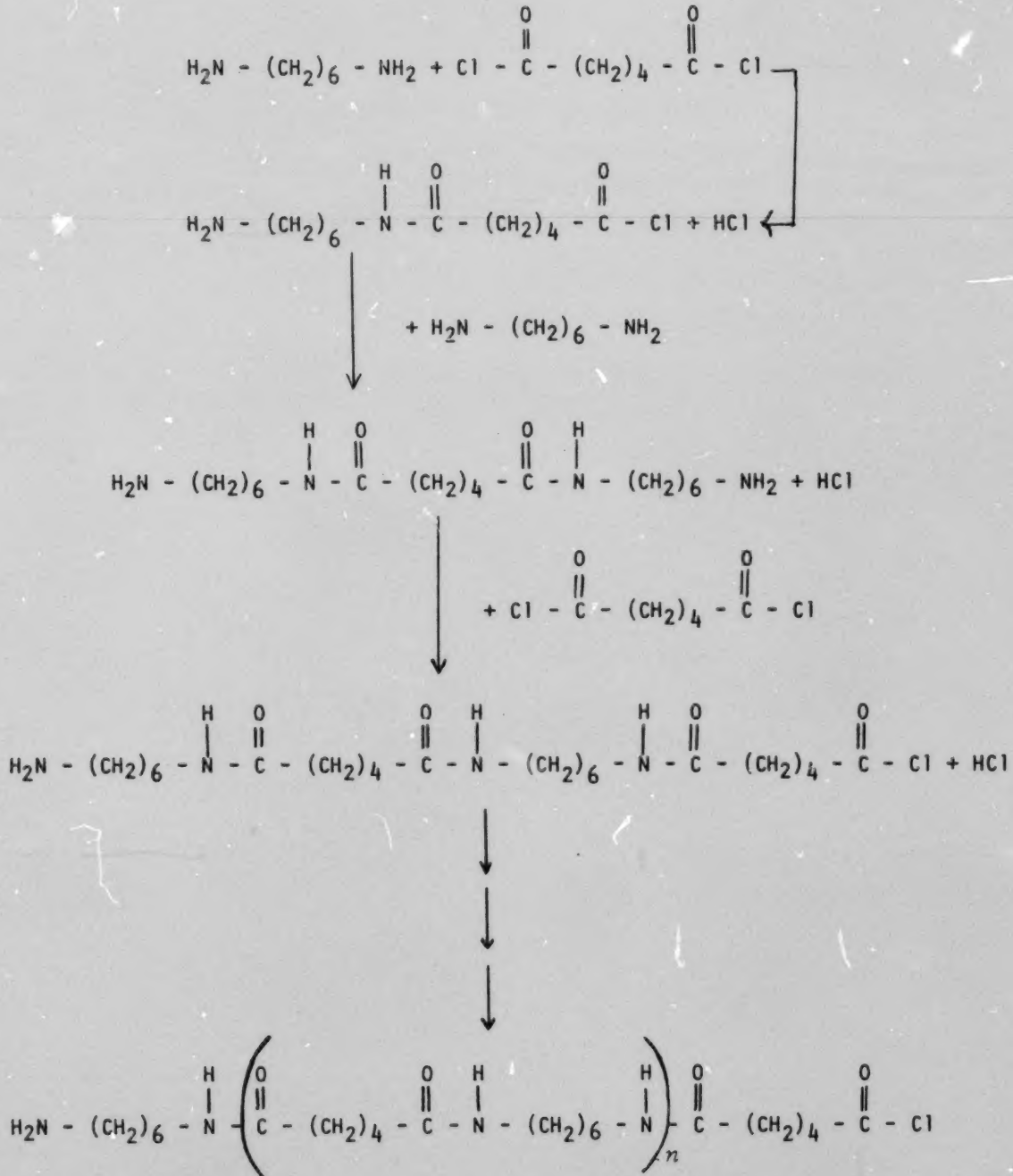


Figure A.1

A.1.a Experiment: PREPARATION OF A POLYAMIDE - NYLON

Work as partners. One partner obtains 25 ml of the dicarboxylic acid chloride solution in a dry 250 ml beaker while the other partner obtains 25 ml of the diamine solution in another 250 ml beaker. Carefully pour the diamine solution down the slanted side of the dicarboxylic acid chloride beaker so that a layer of amine lies under a layer of acid. Using a stirring rod, carefully remove the glob of nylon; raise the polyamide as a rope of continuously forming polymer film to a length of 12 to 15 inches. Cut the polymer at the liquid surface.

A.2 PROTEINS, NUCLEIC ACIDS, CARBOHYDRATES

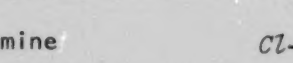
Study of living systems has shown that there are three large polymers of nearly universal occurrence: (1) protein (a polyamide), (2) nucleic acid (a polyphosphoester) and (3) carbohydrate (a polyacetal or a polyketal). Figures A.3, A.4 and A.5 show examples of these three polymers. Each of the types of bonds used to join the four types of monomers is shown in Figure A.2, p. 65.

Some chemistry of acetals and ketals, very important in carbohydrate chemistry, is outlined in Appendix A at the end of the chapter, page 81.

The appendix will help in understanding

This preparation cannot proceed unless the diacid is activated. Therefore the actual reactants in this experiment are a diacid chloride like adipoyl chloride or sebacoyl chloride and a diamine.

adipoyl chloride



sebacoyl chloride

Adipoyl chloride - 5% volume/volume (v/v) 1 ml/20 ml in benzene. Hexamethylenediamine 5% water/volume (w/v) 1g in 18 ml H_2O solution in water with 10 drops of 6M NaOH added to insure that the free amine is present and not amine salt - NH_3^+.

For weight/volume and volume/volume solution, the denominator volume is the final, total volume of the solution. Numerator terms (w or v) are the corresponding measures of the pure solute.

Solution

E.g. a 5% (w/v) of NaCl is 5 g of NaCl dissolved in water to a total volume of 100 ml.

Materials and Equipment

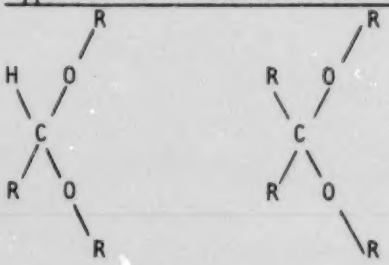
(for one student)

2 beakers (250 ml)
1 stirring rod
1 scissors for whole class
5% (v/v) hexamethylene diamine in water (25 ml)
5% (w/v) diacid chloride in benzene (25 ml)
There are solvent alterna-

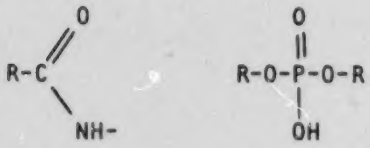
tives for the diacid chloride that may be more available and cheaper: petroleum, ether, ligroin (light naphtha) or gasoline.

Figure A.3 and the discussion of starch and cellulose.

Type of Bond Between Monomers



a. acetal bond b. ketal bond



c. amide bond d. phosphoester bond

Figure A.2

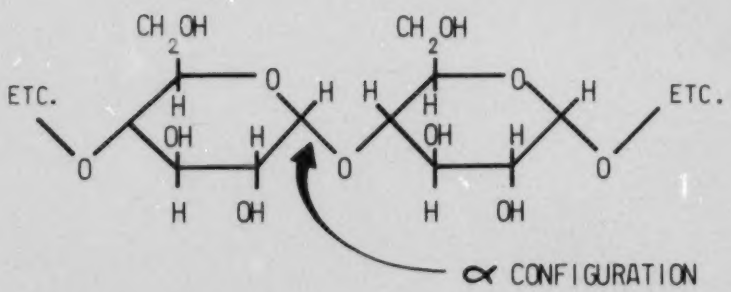
These three polymers--protein, nucleic acid and carbohydrate--lead to an important generalization. Monomeric residues of each biological polymer are linked by a specific type of covalent bond. Thus, those chemical or biological reactions which depend upon the making or breaking of polymers depend upon the making or breaking of specific bond types. Knowing this is a great

aid in the study of living systems, because attention may be focused upon the specific bond type of interest to the investigator.

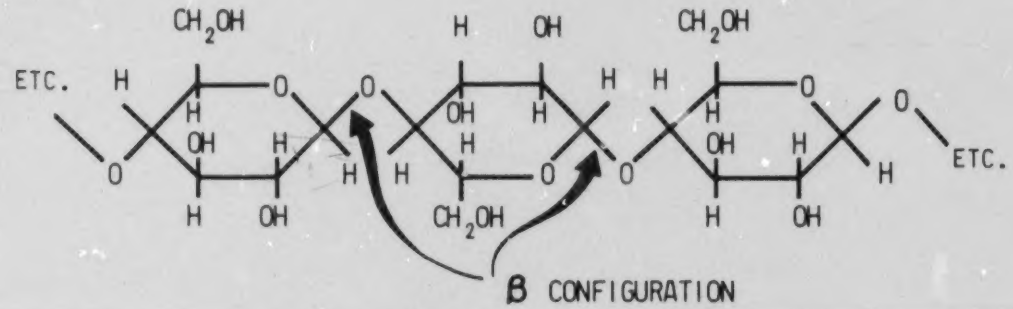
The nature of the bond holding the monomers together is only one factor in the determination of biological activity. It is also possible to influence both the total shape and the biological activity of a polymer by changing the individual monomers in the chain.

Following is an example in which the difference in <u>linkage</u> is important in determining biological activity:

<u>Starch</u> (an α-1-4 linked polymer of glucose)



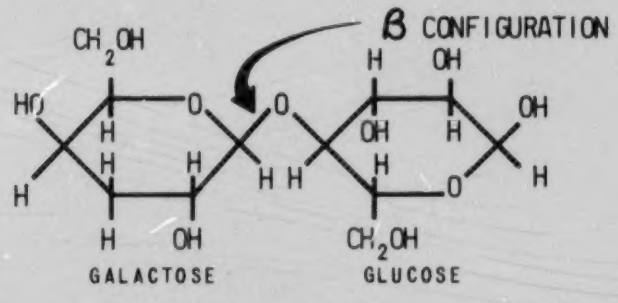
Cellulose (a β-1-4 linked polymer of glucose)



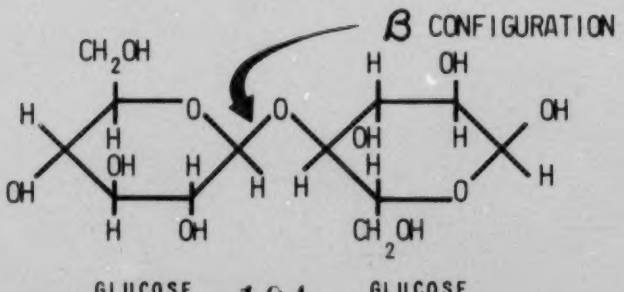
The first polymer, starch, is readily digested by humans and is a staple in our diet. The second polymer, cellulose, is a primary constituent of hay, wood and paper. Cows and termites eat cellulose but depend upon certain microorganisms in their digestive tracts to digest the cellulose (break the polymer down into glucose monomers).

An example where it is the difference in the links (monomers) that determines biological activity follows:

LACTOSE



CELLOBIOSE



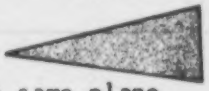
What is the difference between these two structures? Humans digest lactose, a sugar found in milk, but cannot digest cellobiose, a plant sugar.

In the case of starch, each monomer is a glucose molecule and thus each residue in the polymer is the same (see Figure A.3). However, for protein, in which each monomer is an amino acid, there are many different amino acid residues composing the polymer (see Figure A.4).

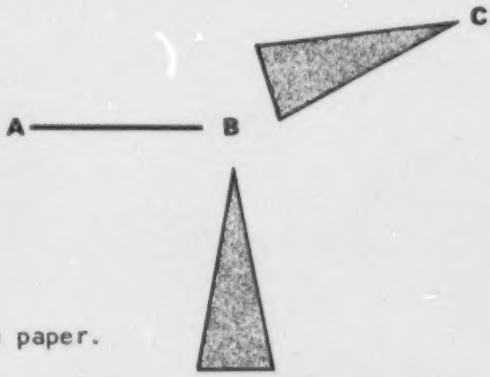
Nucleic acids are also polymers. As in proteins, monomers of the nucleic acid polymers are not all the same. (See Figure A.5). The monomeric residues of nucleic acids are nucleotides (see Figure A.6). A nucleotide is composed of a base bonded to either ribose or deoxyribose, bonded to phosphoric acid. RNA (ribonucleic acid) and DNA (deoxyribonucleic acid) are the nucleic acids containing the sugars ribose and deoxyribose respectively. The number of different bases involved in forming a given nucleic acid isolated from living cells is usually four--a much lower number than the twenty different amino acids composing protein.

If a chain consisting of one hundred links or residues is imagined, it should be obvious that all chains with only one type of link

FOOTNOTE TO FIGURES A.3,4,5

From your work with models you know that three dimensions are required to describe molecules. In Figures A. 3,4,5 an attempt is made to depict three dimensions by use of darkened wedges. A wedge  connecting two atoms means that the two atoms do not lie in the same plane.

For example:



C lies behind the plane of the paper.

D lies in front of the plane of the paper.

A and B are in the same plane - the plane of the paper. (Two atoms joined by a heavy line E-F are both in a single plane in front of the plane of the paper.)

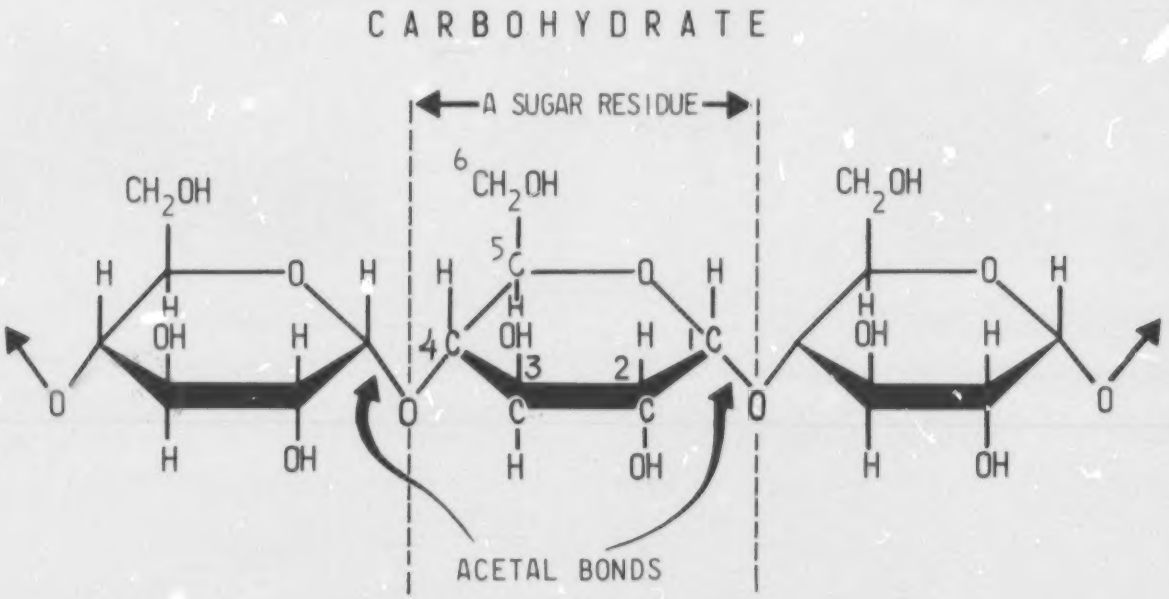


Figure A.3

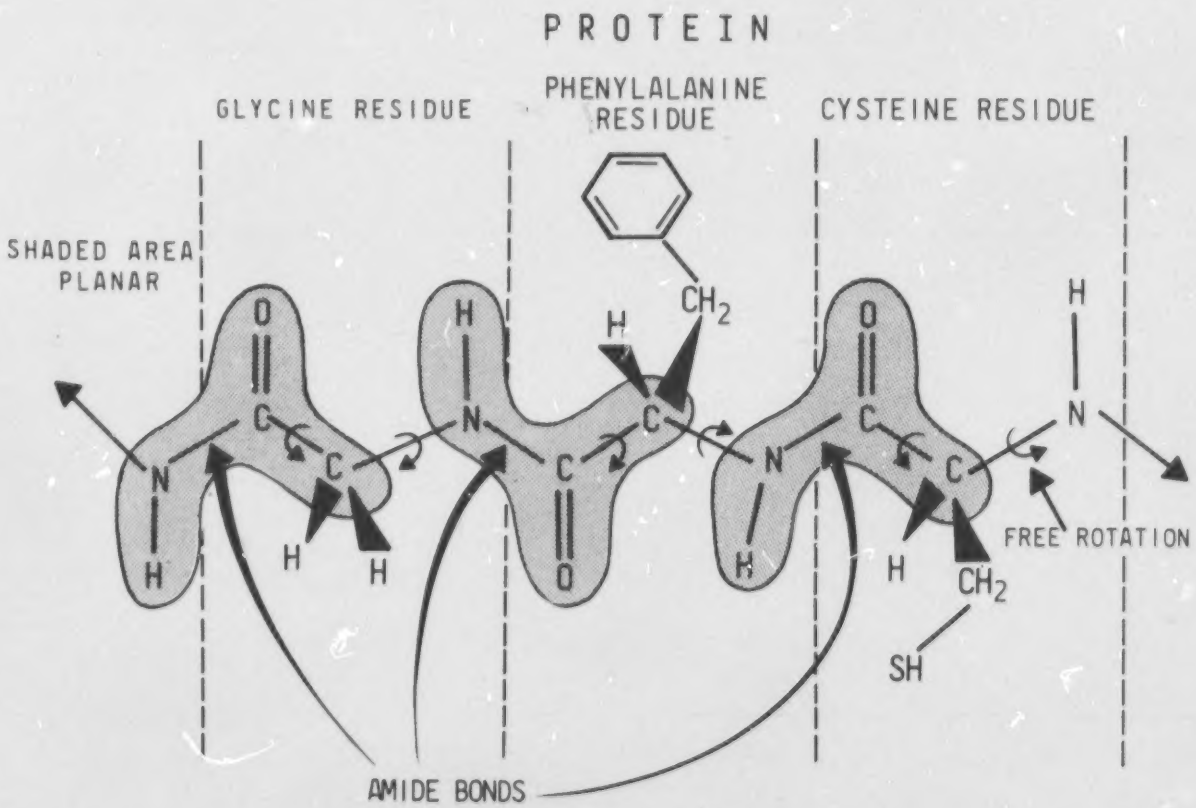


Figure A.4

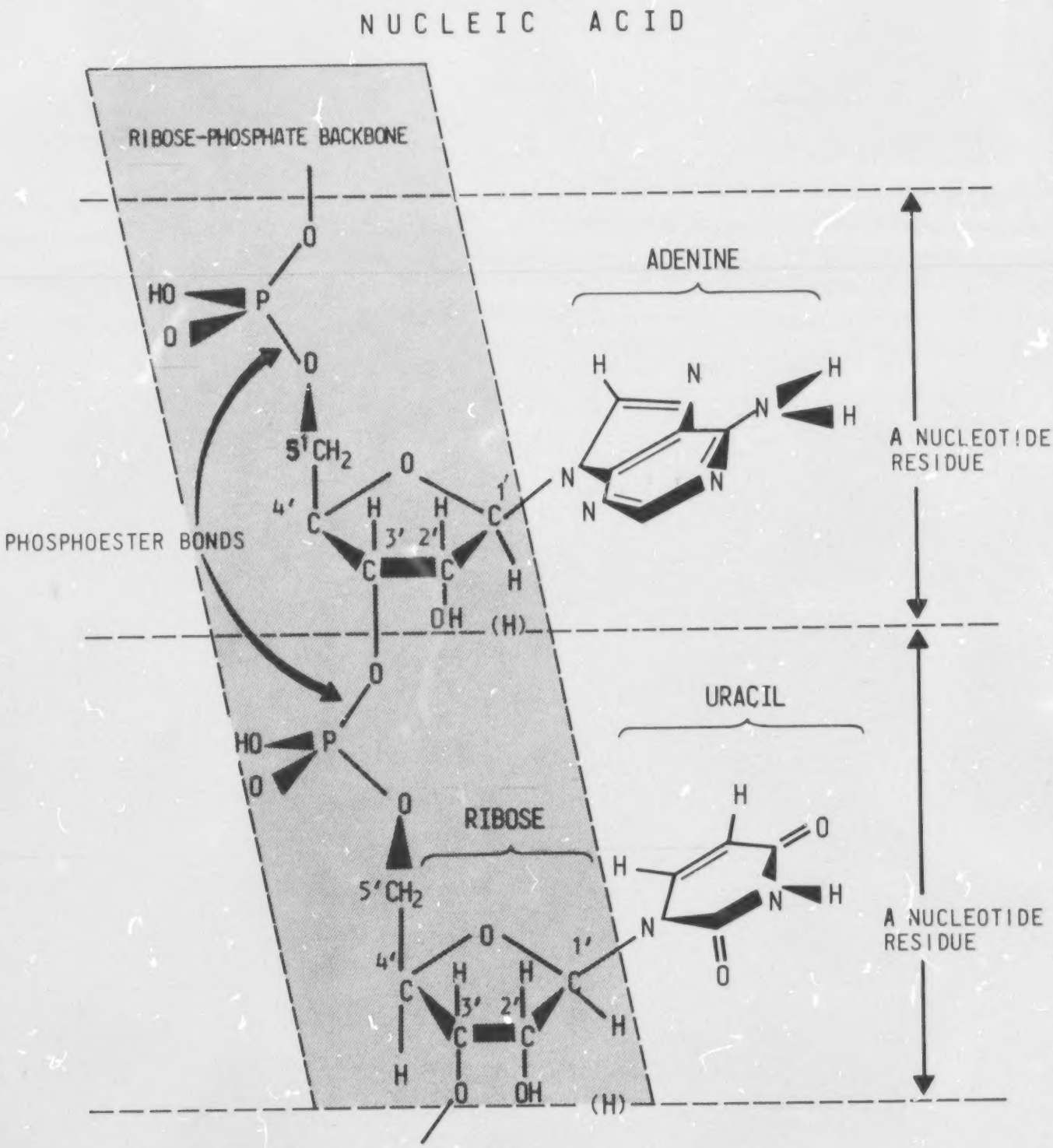
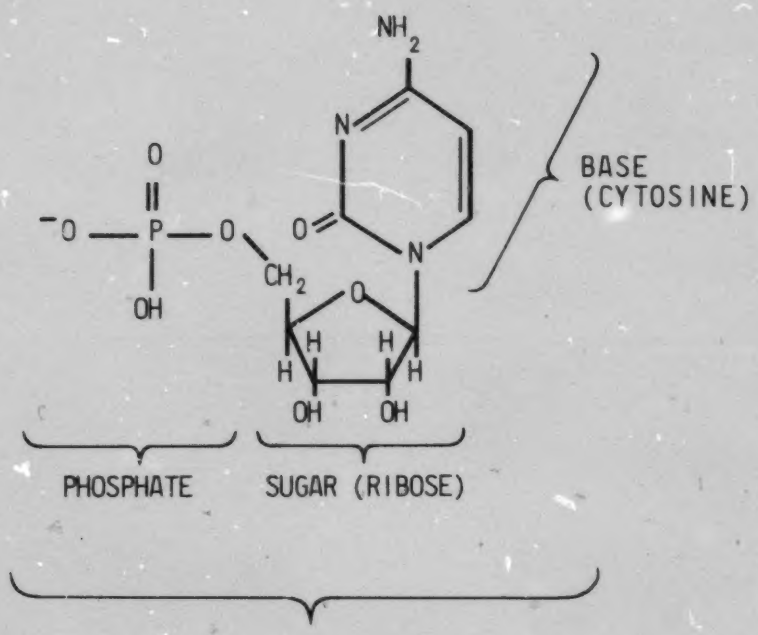
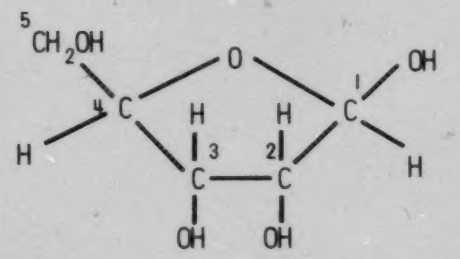
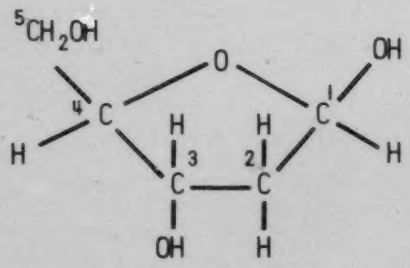

NUCLEIC ACID
RIBOSE-PHOSPHATE BACKBONE
ADENINE
A NUCLEOTIDE RESIDUE
PHOSPHOESTER BONDS
URACIL
RIBOSE
A NUCLEOTIDE RESIDUE

Figure A.5





RIBOSE



2-DEOXYRIBOSE

Figure A.6

(residue) and one type of linkage should be identical. Thus, a starch chain of 100 glucose residues should be identical to all other starch chains of 100 glucose residues. However, a protein chain with differing amino acid residues could lead to an enormously large number of different chains. As an example, a protein 100 residues long composed of 20 different amino acids could have 20^{100} different possible sequences!

Even when the number of different residues is small, there is still the possibility of an enormous number of different possible residue sequences. For example, a nucleic acid 100 residues long composed of only 4 different nucleotides could have 4^{100} different possible sequences.

There are 20 choices for each position in the chain, and there are 100 positions to fill. Therefore there are 20 x 20 x 20 x . . . or $(20)^{100}$ different ways of choosing the sequence

Polymers 100 residues long are big molecules. One of the easiest ways to demonstrate polymer size is to use porous membranes. Two substances, one large and one small, will be placed in a membranous bag. The pores in the bag are large enough to allow the smaller substance in the solution to pass through the membrane but are small enough to retain the larger substance. The membrane is said to be semipermeable. It is capable of separating

the substances.

A.2.a Experiment: POLYMER SIZE

Fill a 400 ml beaker about three-fourths full with distilled water. Cut off a 45 cm piece of dialysis tubing and put it into the distilled water to soak for several minutes to soften it. Securely tie off one end of the tube. Into the tube pour 10 ml each of salt and starch solutions and tie the top closed. Place the bag in the beaker of distilled water.

Instruct students to use masking tape to open the tubing.

A good seal is necessary to prevent the solution inside from leaking out. The safest way is to tie two tight overhand knots in each end of the tube.

Materials and Equipment
(for one student)
1 ml $AgNO_3$ (0.1M)
1 ml I_2 (0.001M)
15 ml NaCl (0.1M)
15 ml Kosher Starch 0.5% w/v
1 beaker 400 ml
1 dialysis tube 45 cm long
4 test tubes 13 x 100 mm

Preparation of Solutions
It is essential for the success of the lab that the starch be Kosher starch, which may be purchased from most grocery stores. This starch is more soluble and gives a better blue color with I_3^- than laboratory "soluble starch." Weigh out the starch, add just enough water to make a paste and work the paste until the starch is thoroughly wet. Add water and boil the starch "solution" for one minute. Cool to room temperature before using. Be sure to refrigerate if stored because starch is rapidly digested by airborne bacteria. The final "solution" may be cloudy, but it will function properly in this experiment.

Stock Iodine Solution (~0.01M

Iodine	*1 g*
Potassium Iodide	*2 g*
Distilled Water to	*300 ml*

Dilute this solution 1:10 to prepare 0.001M solution.

Prepare two test tubes containing 2.5 ml of starch solution and two more test tubes containing 2.5 ml of salt solution. Add several drops of 0.001 M iodine solution to one tube which contains salt. Repeat with a tube which contains starch. To the remaining starch and salt tubes add several drops of 0.1M silver nitrate. Record your observations for each of the four tubes and save the tubes with their contents as standards.

Near the end of the period, remove 5 ml of water from the beaker and divide it equally between two test tubes. Add several drops of 0.001M iodine solution to one tube and several drops of 0.1M silver nitrate solution to the other tube. Observe and record your observations.

The soluble ion is I_3^-. If stoppered, this solution may be stored for years.

Salt particles must have passed out of the bag because the presence of the Cl^- was indicated by the precipitate formed when Ag^+ was added to water removed from the beaker.

I_3^- will pass through the pores in the dialysis bag, turning the starch in the bag blue.

At the beginning of the next day's class again remove 5 ml of water from the beaker and repeat the tests as in the preceding paragraph.

Add several drops of 0.001M iodine solution to the beaker containing the dialysis bag and let it stand for the rest of the period. What happens inside the bag? Explain your observation.

A.3 A CLOSER LOOK AT SIZE

ELemental analysis can be the basis of molecular weight determination. If a chemical compound contains a known element, then the minimum molecular weight can be found by analyzing for the known element. An example is ferrous ammonium sulfate $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$. When the quantity of iron per gram of sample has been determined, it is a simple matter to calculate the grams of sample per mole of iron and thus the molecular weight of the sample. The resulting molecular weight is a minimum molecular weight rather than a true molecular weight because it is expressed on the assumption of a single iron atom per molecule of sample (which is true for ferrous ammonium sulfate). The true molecular weight could be larger by any integral factor,

MW = 392

but it could not be smaller than the determined value.

A.3.a Experiment: POLYMER SIZE - QUANTITATIVE

Obtain 2 ml samples of ferrous ammonium sulfate and hemoglobin solutions from your teacher in separate 250 ml beakers. Slowly add 5 ml of concentrated sulfuric acid to each beaker. Swirl until no particles remain (at least one minute). Add 28 ml of water to each beaker followed by 10 ml of 3% hydrogen peroxide. Swirl until thoroughly mixed. Add a pinch of potassium persulfate, producing a clear, straw-colored solution, then add 5 ml of 3M potassium thiocyanate to each beaker. Filter the hemoglobin solution twice using two sheets of filter paper each time.

Following the directions on page 77 for use of the spectrophotometer, a calibration curve of absorbency vs moles of iron/ml is prepared for various dilutions of the ferrous ammonium sulfate-thiocyanate solution. Dilute the dark red solution with 0.1M potassium thiocyanate (carefully record the amount of liquid added) until a spectrophotometric reading of less than 0.7 absorbency is obtained at 480 mm. Do at least two more dilutions for lower absorbency values, taking readings at each dilution. Plot absorbency on the vertical axis and moles of iron/ml

Materials and Equipment (for one student)

2 beakers - 250 ml
2 test tubes - 13 x 100 mm
1% w/v hemoglobin (2 ml)
1% w/v $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (2 ml)
3% v/w hydrogen peroxide (20 ml)
3M KSCN (10 ml)
0.1M KSCN (50 ml)
Potassium persulfate ($K_2S_2O_8$) 1 pinch
Bausch & Lomb Spectronic 20 (one for the whole class)

on the horizontal axis.

By determining the absorbency of twice-filtered hemoglobin solution, moles/ml of iron in this solution can be read from the calibration curve. From the grams of hemoglobin in solution and its total final volume calculate the grams of hemoglobin/ml.

(1) Divide grams hemoglobin/ml by moles iron/ml.

(2) What assumption must you now make in order to obtain the minimum molecular weight for hemoglobin?

(3) What is the minimum molecular weight for hemoglobin?

(4) How does your experimental value compare to literature values for minimum molecular weight?

(2) Assuming one iron atom per hemoglobin molecule, the quotient obtained in (1) is the minimum molecular weight.

(3) Equals the quotient from (1).

(4) MW hemoglobin 1.6 x 10^4, Handbook of Biochemistry. If the hemoglobin solution is turbid because of improper or insufficient filtering, the absorbency value will be too high and consequently the minimum molecular weight will be too low. In trial experiments the writers obtained a value of 5000 with insufficient filtering and a value of 16,000 when filtering was repeated until a clear solution was obtained.

Students should get detailed instructions on the use of the spectrophotometer from the teacher. Briefly, the general outline is as follows:

These instructions are for a Bausch & Lomb Spectronic 20.

(1) Turn on the instrument (left hand knob) and allow five minutes for warm-up.

(2) Set the zero point (left hand knob) on the transmittance scale with nothing in the sample holder.

(3) Fill one of the spectrophotometer

If any school does not have a spectrophotometer or colorimeter available, then a serial dilution of Fe-SCN complex should be prepared. Simply match the Fe-SCN complex from hemoglobin to one of the tubes of the serial dilution.

tubes with the solvent being used (to be called the blank) and insert this in the sample holder.

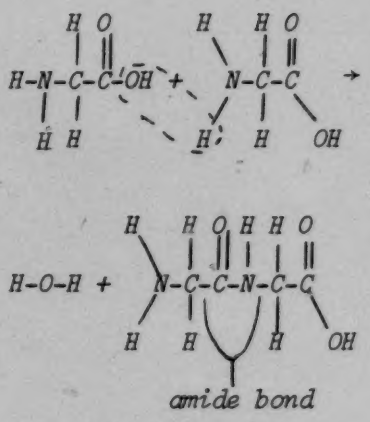
(4) Turn the wave length control to 480 millimicrons and set the absorbency at zero with the light control (right hand knob).

(5) Fill one of the spectrophotometer tubes with sample solution and read the absorbency on the meter.

(6) Repeat step (5) with more or less concentrated sample solutions until enough absorbency values have been collected to span the range from 0.1 to 0.7.

Exercises for Home, Desk and Lab (HDL's)

(1) Draw with structural formulas the reaction of two glycine molecules to produce glycylglycine. Show all atoms and bonds. Designate the amide bond.



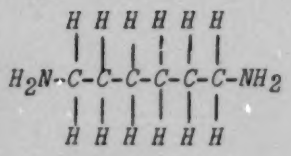
(2) Adipic acid is represented by the structural formula

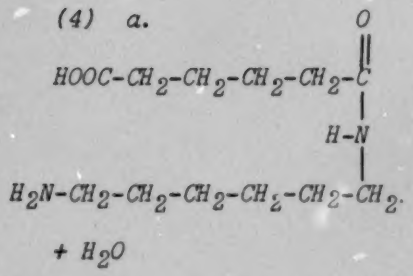
$HOOC-CH_2-CH_2-CH_2-COOH$

a. How many functional groups are present?

b. Name the functional group(s).

(2) a. Two
b. Carboxyl (both)

(3) 

(4) a. 

b. *Amide or peptide bond.*

(5) a.

b.

(6) a.

b.

c.

d. *Two*

(7)

(8) a.

b.

c.

d. *Three*

(3) Draw the structural formula for 1, 6-diamino hexane.

(4) a. Draw the structural formula for the product of the chemical reaction between adipic acid and 1, 6-diamino hexane.

b. What is the type of bond that holds the monomers together?

(5) Draw the functional group

a. for an aldehyde;

b. for an alcohol

(6) Write the structural formula for:

a. ethane

b. ethanol

c. ethanal

d. How many carbon atoms are in each molecule?

(7) What is the ketone functional group?

(8) Write the structural formula for:

a. propane

b. propanol

c. propanone

d. How many carbon atoms are in each molecular formula?

(9) Write the equation for the chemical reaction between two moles of propanol and one of propanone.

(10) What distinguishes an acetal from a ketal functional group?

(11) Use your model kit to prepare the molecule formed in question (9).

(12) Draw the structural formula for phosphoric acid.

(13) Draw the functional group for:

a. an acid

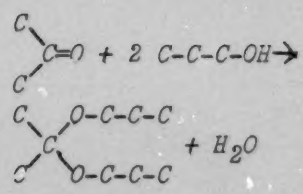
b. an ester

(14) What feature distinguishes an acid from an ester?

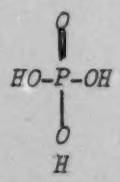
(15) a. Draw the methyl ester of phosphoric acid.

b. What is the name of the -O-R bond in the molecule shown in (15) a?

(16) Use your model kit and make the molecule in problem 15.

(9) 

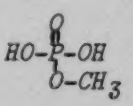
(10) The carbon atom of the acetal has a hydrogen attached to it, whereas the ketal carbon atom is attached to carbon atoms only.

(12) 

(13) a. COOH

b. COOR

(14) The -OH of the acid is replaced with an -OR in the ester.

(15) a. 

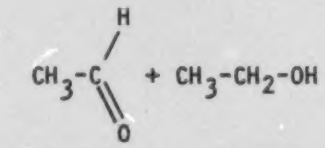
b. Phosphoester bond

TEXT SECTION	ROUGH TIME ESTI-MATES	EXPERIMENTS	DEMONSTRATIONS	TEACHING AIDS	OTHER STUDENT ACTIVITIES	PROBLEMS	OUTSIDE READING
Chap IV. Polymers in 3D, or shape of things to come A. Polymer shape	1 1/2 Days	A.1 Polymer shape or permanent wave		Models		1, 3-5	The Double Helix, J. D. Watson Signet 03770 1968
B. Weaker bonds B.1 H-bonds between bases B.2 Hydrophobic bonds between bases C. The importance of shape	1 1/2 Days			Journal of Biological Chem Vol 243, p. 1664, 1968 Scientific American, p. 78 Nov. 1966		6-11	Scientific American Oct. 1954 Sept. 1957 May 1967

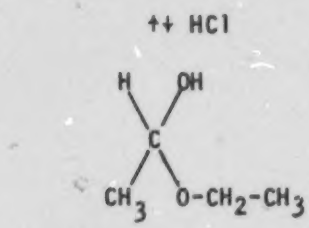
80a

APPENDIX A

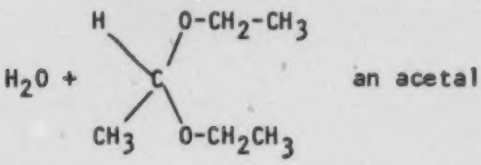
Acetals are prepared from alcohols and aldehydes:



ethanal (aldehyde) + ethanol (alcohol)

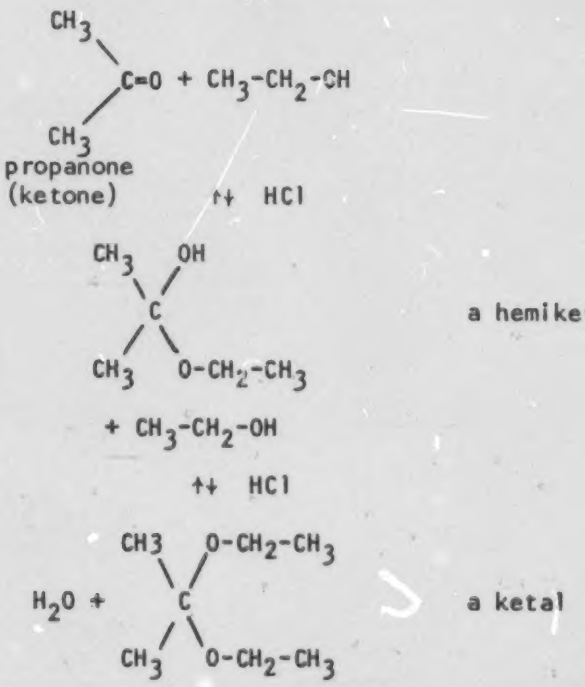


a hemiacetal



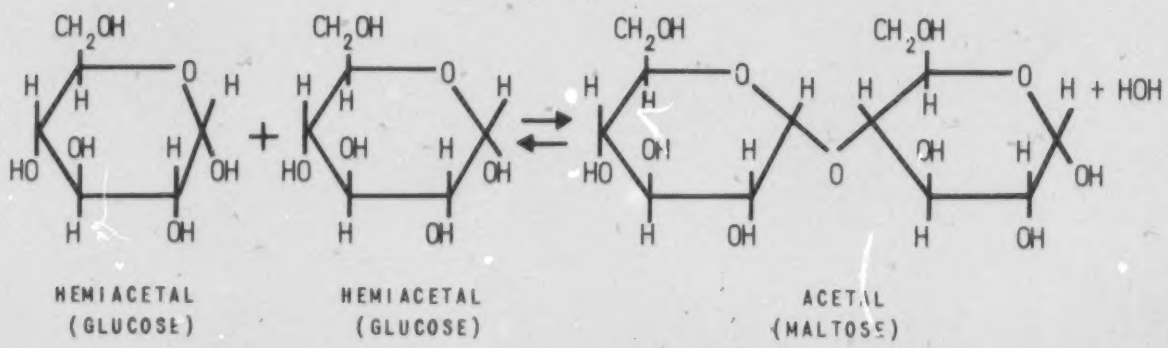
an acetal

Ketals are prepared in the same way using alcohols and ketones:

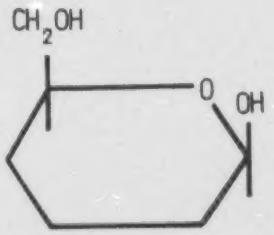


The distinguishing feature of an acetal is the hydrogen attached to the former carbonyl carbon. A ketal has no hydrogen attached to this carbon.

The most common biological instance of polyacetals involves the reactions of a hemiacetal with another hemiacetal.

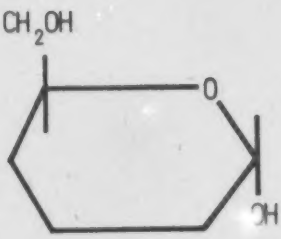


If the hydroxyl of the hemiacetal or hemiketal is on the same side of the ring as the last carbon (number 6 for hexose, number 5 for pentose), the configuration of the hemiacetal or hemiketal is called β.



β CONFIGURATION

The opposite configuration is called α.



α CONFIGURATION

The same convention is used for acetals and ketals, as well as for other functional groups.

Chapter IV: POLYMERS IN 3D OR THE SHAPE OF THINGS TO COME

A. POLYMER SHAPE

The long chain-like molecules that have been discussed have specific three-dimensional shapes. It is easy to imagine possible ways of arranging a chain in space: stretched straight; coiled like a spring; randomly tangled; or neatly rolled into a sphere. The number of possible arrangements is enormous.

Factors which determine polymer shape are the possibilities of bonding between various portions of the polymer and the relationship between the polymer and its environment. If bonding is a factor, then there will be a range of bond strengths. This range of bond strengths is given in Table A.1.

Relative Bond Strengths

Type of Bond	Van der Waals	:	Hydrogen	:	Covalent
Kcal/mole	1	:	10	:	100

Table A.1

The Scientific American article (March 1968) on insulin structure gives a good example of molecular shape as determined by disulfide bonds. Some classes have constructed the insulin molecule using approximately 8 FMM kits.

The strongest bond listed is a covalent bond. An important covalent bond in determining protein shape is the disulfide bond (-S-S-) formed between two molecules of the amino acid cysteine ($HS-CH_2-\underset{NH_2}{CH}-COOH$). Hair is a protein with a high cysteine content. It is built up of two or more polypeptide chains cross-linked through disulfide bonds.

$-S-S- + 2[H] \longrightarrow 2\ -S-H$
reduction

Treatment of hair with permanent wave lotion reduces the disulfide bonds to sulfhydryl (-SH) groups. Destruction of the disulfide cross-link permits stretching and rearrangement of the protein molecules. This is called curling the hair. Following curling, new disulfide bonds are formed by oxidation. Permanent hair curling results from pairing of different sulfur atoms form those which were joined in the original hair.

$2-S-H \longrightarrow -S-S- + 2[H]$
oxidation

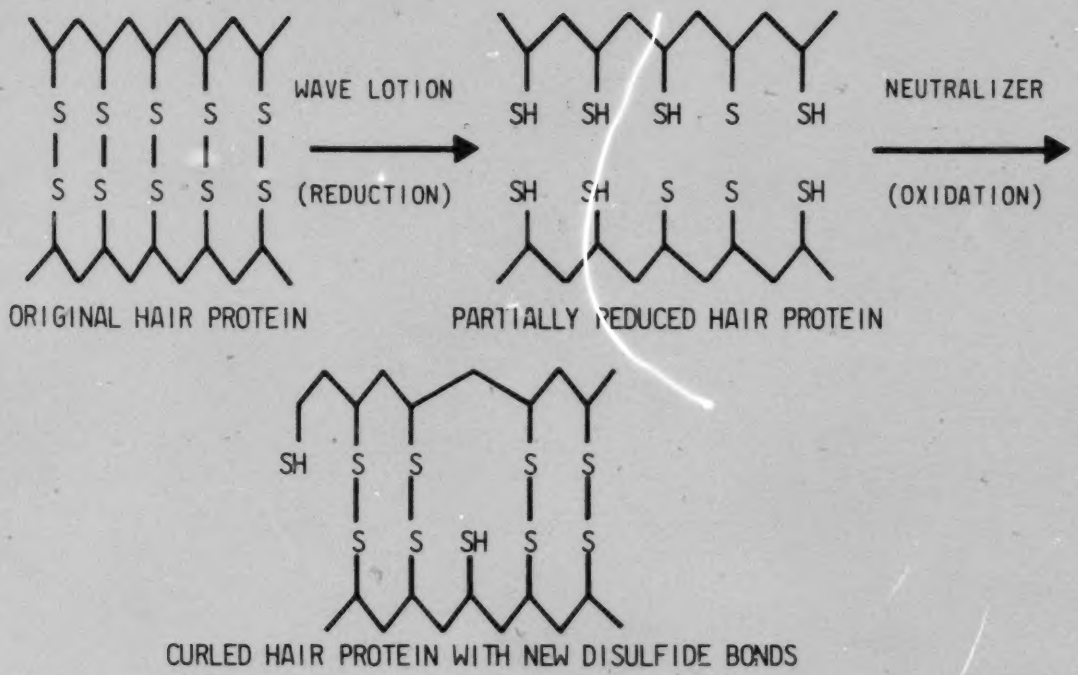


Figure A.2

A.1 Experiment: POLYMER SHAPE OR PERMANENT WAVE

Obtain two strands of long, straight hair. Wind each strand of hair around a glass stirring rod. Hold the hair in place upon the stirring rod with rubber bands. Wash the hair by vigorously swirling the hair-wound stirring rod in a beaker of soapy water. Thoroughly rinse the hair under running water. Immerse one rod of hair in permanent wave lotion contained in a test tube. Immerse the other rod of hair in an

After long, hard thought the writers were unable to come up with a more effective experiment than this inexpensive and graphic demonstration.

equal volume of water in another test tube. Set the two tubes aside for 15 minutes. After the 15-minute interval, remove the two hair-wrapped rods and rinse with running tap water until the odor of lotion cannot be smelled. "Neutralize" (oxidize) the hair following the directions of the specific commercial product used.

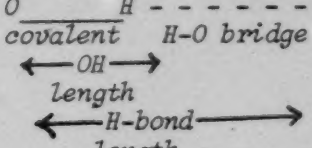
B. WEAKER BONDS

B.1 H-BONDS BETWEEN BASES

Weaker bonds, for example hydrogen bonds, were discussed in Chapter 1 of Chemistry of Living Matter and Chapter 17, CHEM Study. One important factor in determining the three-dimensional shape of nucleic acids is hydrogen bonding between bases. Two chains of nucleic acid polymers are held together by hydrogen bonding between bases. Construct models of the four bases of DNA: thymine, adenine, cytosine and guanine. Can these bases hydrogen-bond to each other? Try it with models.

The most stable arrangement would be the one with the most hydrogen bonds. Since there are four different bases, how many different hydrogen-bonded pairs are possible? Which arrangements of base pairing would you predict to be most stable? Compare the sizes of the hydrogen-bonded pairs in which you find maximum

Any odd colored tubing will do. The length of a hydrogen bond is from 2.8 to 3.0 Å. i.e.:



With four different bases there are 10 different hydrogen-bonded pairs possible.

Those base pairings which maximize the number of hydrogen bonds between

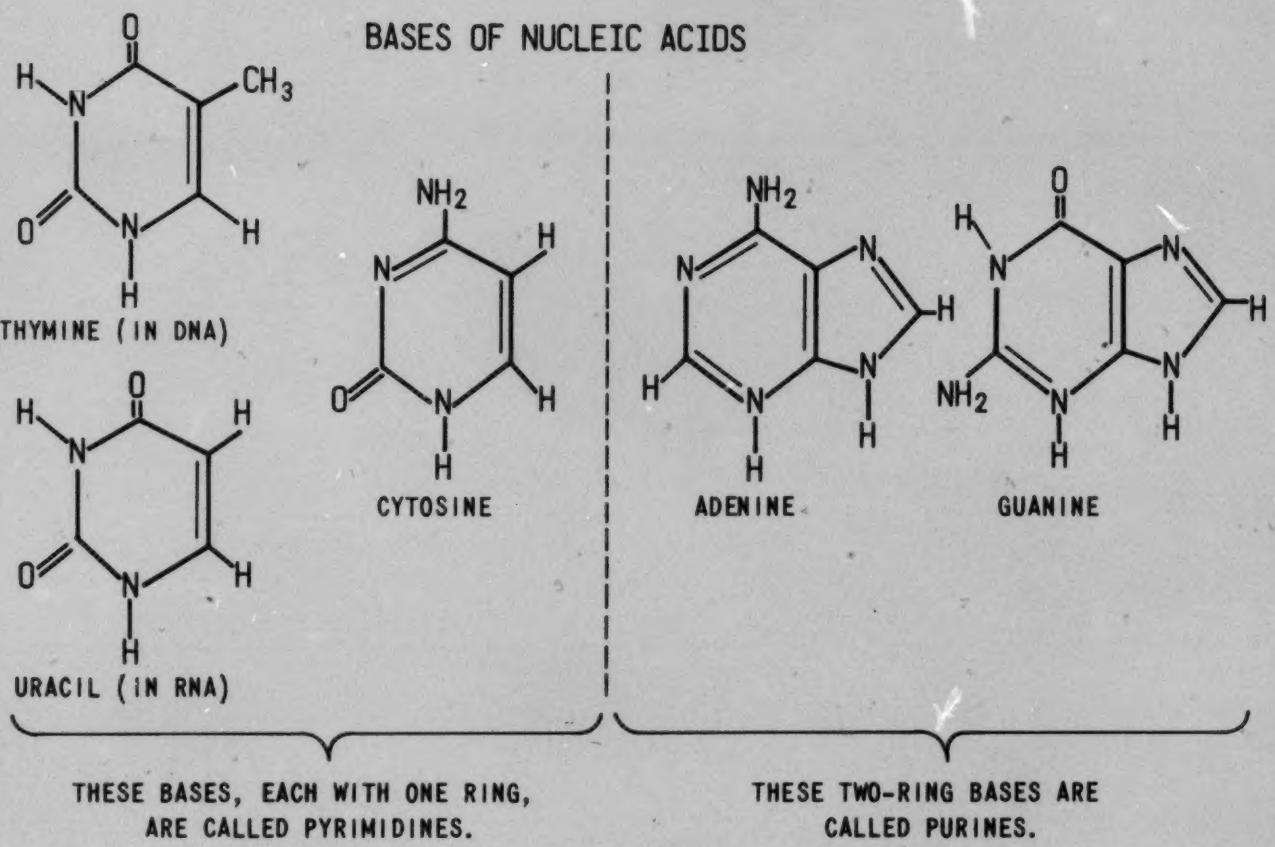


Figure B.1

hydrogen bonding. Pairing of smaller with larger gives a constant-sized pair. Refer to Figures B.2 and B.3.

If the purines and pyrimidines of two separate DNA chains are paired by hydrogen bonding *bases are most stable, and they are shown in Figures B.2 and B.3.*

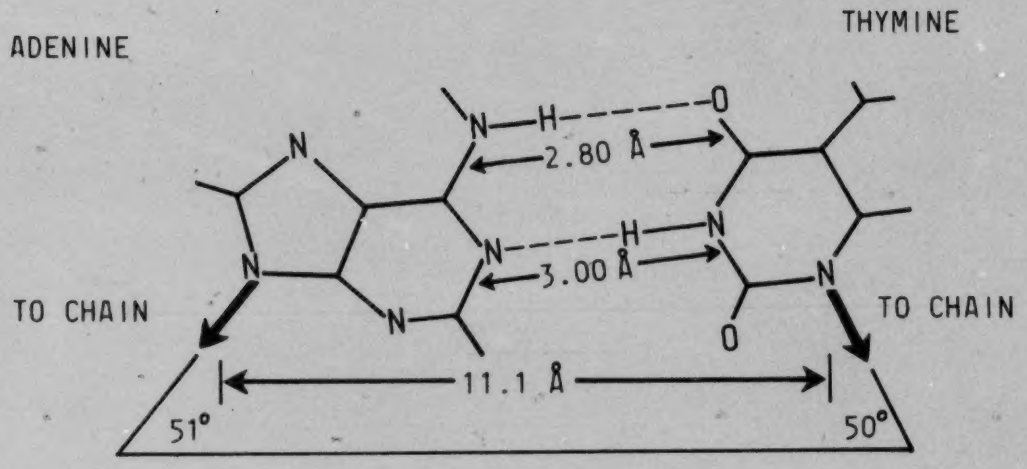


Figure B.2

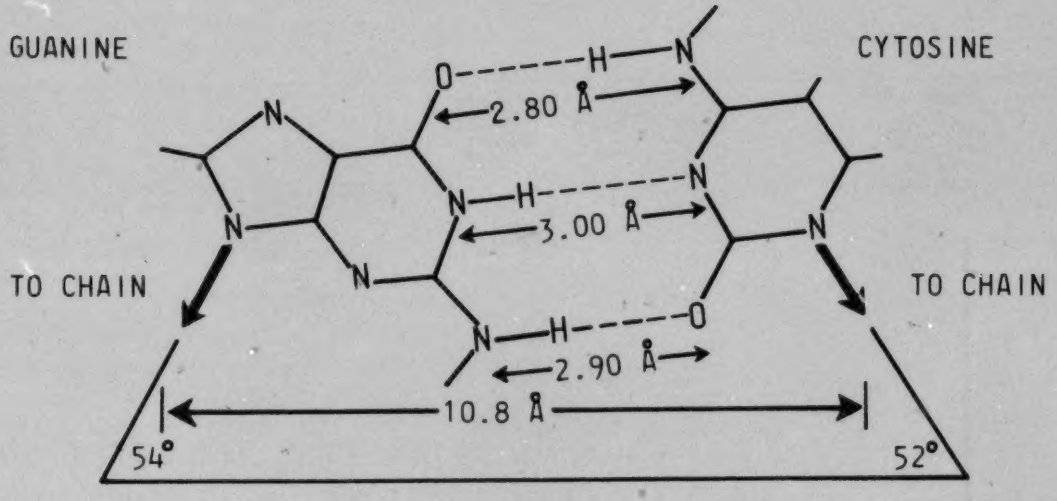


Figure B.3

along their edges, as in Figure B.4, then the two DNA polymers would be held together.

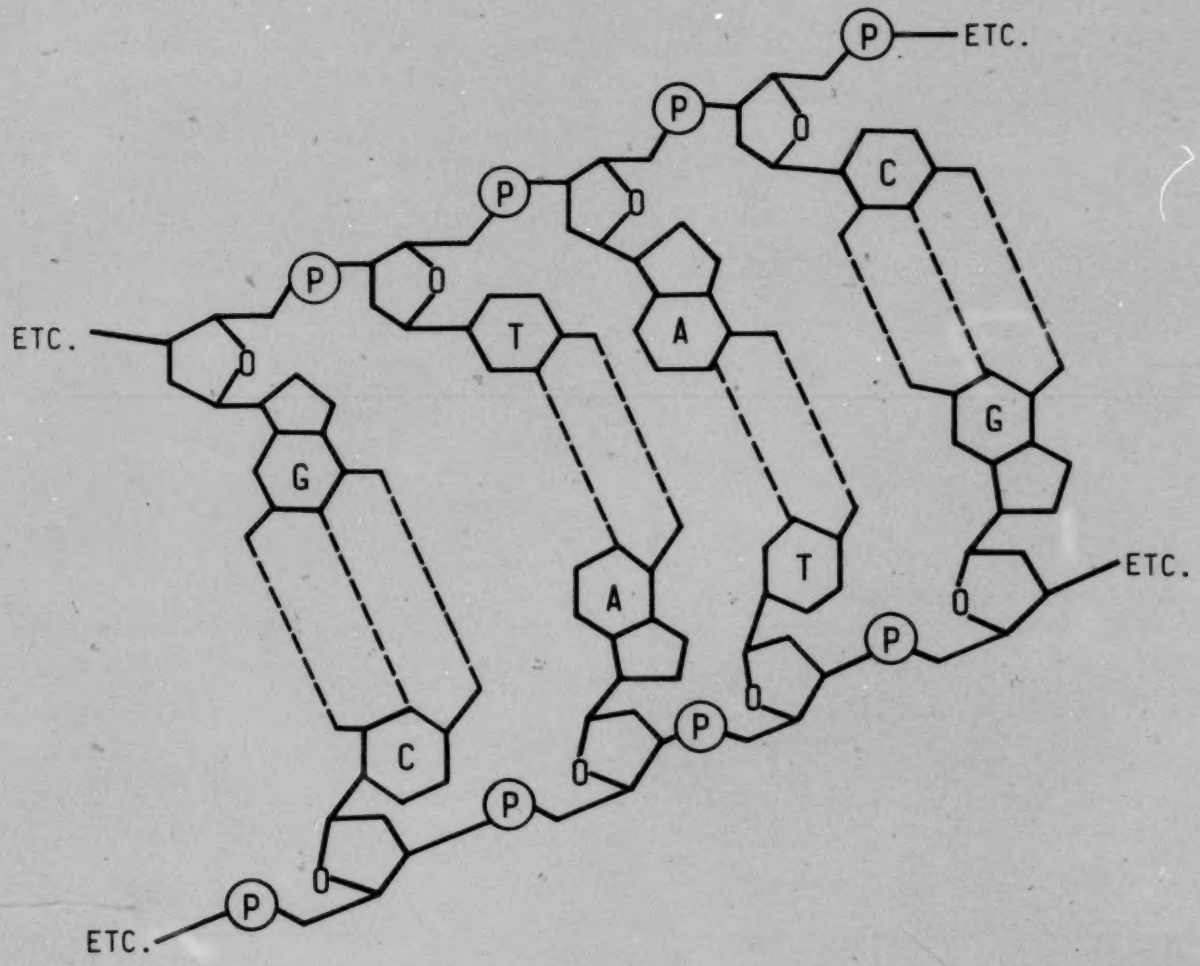


Figure B.4

Two DNA chains joined in this fashion are called complementary chains. Every base in one chain must be joined by hydrogen bonding to its unique complementary base in the other chain. Thus DNA is double stranded rather than single stranded because of hydrogen-bonded base pairing.

B.2 HYDROPHOBIC BONDS BETWEEN BASES

Until quite recently there has been a tendency to neglect the role of water in determining three-dimensional shape. The nitrogen and oxygen atoms of amide, acetal and phosphoester bonds can hydrogen-bond to water. However, there is another important role of polar water molecules.

Insertion of hydrocarbons into water has an organizing effect upon the surrounding water molecules. In effect an "ice sheath" is formed around each hydrocarbon molecule, and the water molecules in these sheaths are not as free as those elsewhere in the solution. This leads to a decrease in randomness, thus an increase in usable energy of the system. If the hydrocarbon residues of polymers can fold upon themselves, decreasing the surface area exposed to water, then organization of the surrounding water is reduced. This leads to an increase in randomness of the water molecules, thus a decrease in usable energy; this is, of course, what happens. This process is called "hydrophobic bonding."

Remember that $\Delta G = \Delta H - T\Delta S$ where ΔH is the enthalpy of the system and ΔS its entropy. ΔG, the Gibbs free energy, is the maximum usable energy in the system. Also recall that $\Delta G=0$ for a system at equilibrium. For the system illustrated here it is true that in the process of hydrophobic bonding the increase in randomness more than offsets the heat required to melt the "ice-structures" surrounding hydrocarbons. That is to say that when $\Delta G = \Delta H - T\Delta S<0$, hydrophobic bonding occurs spontaneously, leading to the equilibrium situation.

The DNA backbone, on the other hand, is very soluble in water. The -OH groups on ribose and the charged phosphate groups both react strongly with water.

The purine and pyrimidine base residues in DNA are quite insoluble in water because their non-polar, large, flat sides will not hydrogen-bond even though they contain polar C=O and

-NH_2 groups on their edges. "Hydrophobic bonding" of DNA's flat purine and pyrimidine residues would cause them to stack like playing cards. We would predict DNA would assume either a zigzag or helical conformation in order to fold up the backbone and bring the base residues close enough to stack.

In this respect the DNA structure is "schizophrenic," half hydrophobic and half hydrophilic, and this is undoubtedly very important in the final structure of the molecule.

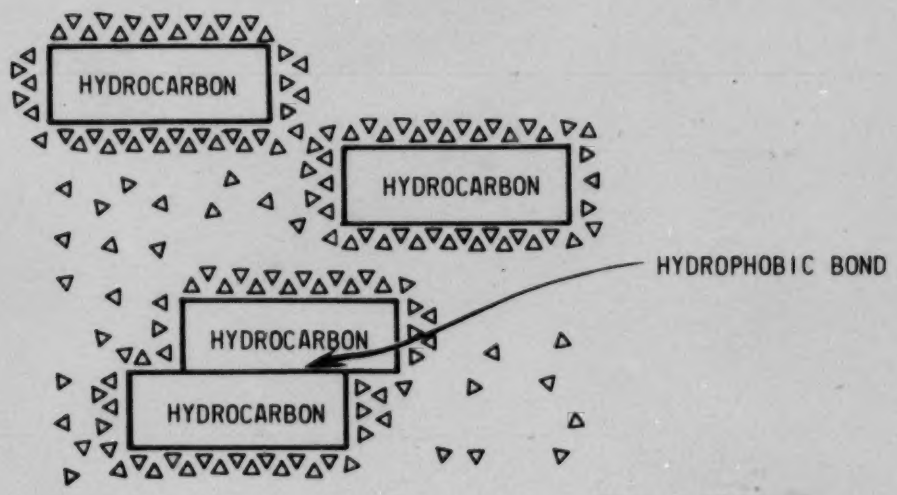


Figure B.5 - Hydrophobic Bond Formation. The triangles represent water molecules. As the hydrocarbon residues approach each other some of the water molecules are released from an ordered arrangement close to hydrocarbon to a more disordered state in the solvent.

In fact, DNA molecules have regular helical shapes (like a "slinky"). The stacked base pairs, which are all of equal size, require that the shape of a DNA molecule be a regular helix. This means that the helix has a constant diameter and a constant number of turns per unit length (constant pitch).

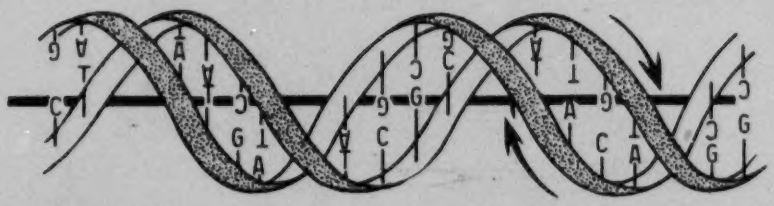


Figure B.6

The preceding discussion on the shape of DNA is more elegantly and more powerfully presented in the words of James D. Watson. For his description of his discovery of the DNA double helix structure see pages 111-112, The Double Helix (reprinted in the Feb. 1968 Atlantic Monthly).

The Double Helix, J. D. Watson, Signet Q 3770, (1968).

C. THE IMPORTANCE OF SHAPE

There has been a continual emphasis in this section upon the importance of the three-dimensional shape of biological polymers. Despite the fact that structural determination of large molecules is difficult, the three-dimensional shape of several large molecules has been determined. The three-dimensional representation of the protein lysozyme, taken from the Journal of Biological Chemistry, Volume 243, p. 1664, 1968, should be compared to the representation of the same molecule in Scientific American, November

1966, page 78. The deficiencies of such pictures are obvious, leading to the model-building activities of many people engaged in polymer research.

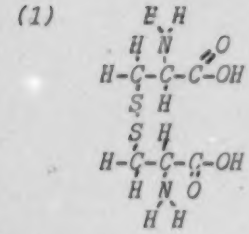
The pictures of DNA in Scientific American, October, 1954 (Crick), September, 1957 (Crick), and May, 1967 (Yanofsky), are more adequate than the lysozyme pictures for visualizing structure because double-stranded DNA is more regular in its structural features.

The idea of complementary base pairs can be expanded to the broader idea of complementary surfaces. The idea of complementary surfaces is important in understanding the 3-D shape of polymers. Weak forces are effective only when the interacting surfaces are close. This closeness is possible only when the molecular surfaces have complementary structures. For instance a bump or a positive charge on the surface of an enzyme must be paired with a hole or negative charge on the substrate surface. Biochemists have used the phrases lock-and-key relationship or template relationship to describe these complementary surfaces. We shall try to establish a connection between complementary surfaces of biological polymers and biological activity.

Hydrophobic bonding is extremely important in the structure of globular proteins such as lysozyme or hemoglobin. Unfortunately proteins lack the simple sequence regularities of DNA and it is extremely difficult --or impossible--to predict a priori exactly how such bonding will influence the resulting protein structure However, it is a rule of thumb among protein chemists that most globular proteins have a "grease-pit ," i.e. a cluster of hydrophobic amino acid residues which form the core of the structure.

While ionic (charge-charge) interactions decrease as $1/r^2$, the weak van der Waals forces decrease approximately as $1/r^6$; obviously close proximity is required for such interactions.

(1)



(2) *Amide, phosphoester, acetal, ketal and disulfide.*

(3) *Hydrogen bond and hydrophobic bond.*

(4) *Bonding possibilities between the various portions of a polymer and the relationship between the polymer and its environment are the factors which determine polymer shape.*

(5) *Oxidation of sulfhydryl bonds on hair, while curling, produces new disulfide bonds which cross-link the protein of the hair when wound on the curler.*

(6) *See text.*

(7) a. *Purines adenine and guanine bond with pyrimidines thymine and cytosine respectively.*

b. (1) *Adenine and thymine have two hydrogen bonds.*

(2) *Guanine and cytosine have three hydrogen bonds.*

c. *A-T has hydrogen bonds between the N and O atoms and*

Exercises for Home, Desk and Lab (HDL's)

(1) Draw the structural formula for two molecules of cysteine connected by the covalent disulfide bond. Then construct this molecule with your kit.

(2) Name five covalent bonds in biomolecular molecules.

(3) Name two weak bonds that hold molecules together.

(4) What factors determine polymer shape?

(5) What role does oxidation play during treatment of hair with a permenent wave lotion?

(6) Diagram the structure of the two purine bases and the three pyrimidine bases. Name each diagram.

(7) a. Which purines pair with which pyrimidines?

b. Identify the number of hydrogen bonds between each base pair.

c. Hydrogen bonds exist between what two atoms in each base pair?

(8) Hydrophobic bonding describes the situation which exists between hydrocarbons and their water environment. How is water organized in a sheath when hydrocarbons are placed in water?

(9) What spatial arrangement would you predict

for purines and pyrimidines, knowing that such hydrocarbons undergo hydrophobic bonding in a watery environment such as the cell?

(10) The DNA backbone is composed of linkages between what molecules?

(11) Would one expect a single strand of DNA to form a helix or are both strands necessary? Provide the reason for your expectation.

N and N atoms. G-C has hydrogen bonds between two sets of N and O atoms and one set of N and N atoms.

(8) When hydrocarbons are inserted into water the water is organized in a sheath around the nonpolar portion of the hydrocarbon since polar and nonpolar molecules are not compatible.

(9) Hydrophobic bonding between the purines and pyrimidines flat sides (top and bottom) cause the bases to stack like playing cards. This stacking, along with the phophodiester linkages in the backbone of DNA, causes DNA to develop a helical conformation.

(10) The mononucleotide units are linked together through phosphodiester bridges between the deoxyribose portions of successive nucleotides.

(11) A single strand of DNA could possibly form a helix by itself because of the stacking due to hydrophobic bonding between the flat purine and pyrimidine residues and because of the folding up of the backbone due to phosphodiester bridges.

TEXT SECTION	ROUGH TIME ESTI-MATES	EXPERIMENTS	DEMONSTRATIONS	TEACHING AIDS	OTHER STUDENT ACTIVITIES	PROBLEMS	OUTSIDE READING
Chap. V Where the action is		A.1 Proteolytic enzymes		Models	Students bring to class common household items that may contain proteolytic enzymes.	1, 5	
	5 Days	A.2 Saliva and its substrate		Film: CHEMS Mechanism of organic reaction			
		A.3 Specificity of urease		Film: CHEMS Biochemistry and molecular structure		2, 3, 6	

97

Chapter V: WHERE THE ACTION IS--THE ACTIVE SITE

All known biological catalysts are proteins called enzymes. Each enzyme catalyses a specific chemical reaction that is needed by the living organism. The material on which the enzyme operates is called the substrate. Enzymes are often named by adding ase to the name of the substrate.

Proteolytic enzymes are those that digest (break down into amino acids) other proteins. These are widespread in nature, being found in bacteria and higher plants as well as in the digestive systems of animals. Some of these proteolytic enzymes have been useful in industry and commerce, and a few are found in common household or kitchen items. Can you think of some such items?

A. LAB ACTIVITIES WITH ENZYMES

A.1 Experiment: PROTEOLYTIC ENZYMES

Bring to class samples of any common product or food item which you think may contain proteolytic enzymes. An easy test for these enzymes is through their digestion of gelatin (the protein used in gelatin desserts). For this test you will use a solution of gelatin which has solidified into a jelly. In this jelly, gelatin molecules form a three-dimensional net-

Usually they will digest themselves as well as other proteins, so that solutions of proteolytic enzymes often will lose their activity in a few hours.

Students should come up quickly with meat tenderizer and enzyme detergents. Less obvious will be such things as rennet or junket-type dessert and such fresh fruits as pineapple or papaya. Students should be encouraged to bring small samples of these items and anything else they wish to test, e.g., saliva and the enzyme urease (to be used in a succeeding experiment).

Gelatin is a soluble form of collagen--a major protein component in bones and cartilage.

Materials and Equipment
(for one student)
One to three gelatin plates. Soak 50 gm of Knox's plain gelatin in 50 to 100 ml of cold water. Add to this 900-950 ml of boiling water and stir to dissolve. Then distribute 15 to 20 ml volumes into little plastic petri dishes (Shaw Surgical Co., Portland) and allow these to set at room temperature. You should obtain

about 50 plates from a liter of gelatin solution.

The holes in the gelatin will probably become obvious in six to eight hours. The teacher may wish to refrigerate the plates at this point until the next class period.

The proteolytic enzymes in some common items are

item	*enzyme*
fresh papaya	*papain*
meat tenderizer	*papain*
fresh pineapple	*bromelin*
enzyme detergents	*bromelin or subtilisin*
rennet or Junket dessert	*rennin*

In addition, if available from the local slaughterhouse, fresh blood, gastric juice and the pancreas all contain proteolytic enzymes.

Materials and Equipment
(for one student)
4 test tubes
(13 x 100 mm)
3 droppers
iodine - KI solution (1 ml)
0.5% soluble starch solution (1 ml)
Meat tenderizer (pinch)
Phenolphthalein indicator solution

Iodine - KI solution: Dissolve 1.5 g KI in 25 ml water. Add 0.3 g I_2; stir until dissolved. Dilute to 150 ml with water.

0.5% soluble starch solution: Kosher potato starch is better then laboratory soluble starch. Add 0.5 g starch to 5 ml of

work with the water trapped in this network. Any enzyme that digests gelatin will break down this network, thereby dissolving the jelly. Try your samples of meat tenderizer, enzyme detergent, etc. Spot a few grains of each onto the surface of a gelatin plate and add a drop of water to each spot. You should also try a drop of saliva and any other materials available. Be sure to label the plate with your name and also (on the back) with the names and location of each material spotted. Let the plate stand at room temperature overnight. Then gently wash the surface of the plate under tap water and gently shake the water off the surface. Have any of your samples "eaten" holes in the gelatin?

A.2 Experiment: SALIVA AND ITS SUBSTRATE

Collect a one milliliter saliva sample in a 13x100 mm test tube. Add one drop of starch solution to the saliva and mix the test tube by shaking. Set the tube aside. After at least ten minutes, add one drop of iodine solution to the test tube containing saliva. Shake the tube and record the color of the contents of the tube.

Repeat the above procedure, omitting the ten minute delay. Record the color of the contents of the tube.

Add a pinch of meat tenderizer to a 13x100

enzymes. Don't dwell on competitive inhibition" when talking to students but rather er emphasize the idea. After seeing how urease catalyzes the hydrolysis of urea but not thiourea or acetamide ask if the students can explain the lack of reaction in the latter two cases. Based on the film, their answers might be that the incorrect substrate could not fit into the active site or else, once fitted in, the reaction could not take place. Ask them how we might check these two theories. If the substrate would fit into the active site but not react to be hydrolyzed, then there would be no active enzyme in the thiourea or acetamide test tubes (provided that an excess of thiourea or acetamide were used). If, however, the molecules would not fit into the active site, the presence of active urease should be capable of detection by the addition of urea to the test tube. The phenolphthalein in the test tube would turn pink due to the liberation of ammonia.

In doing tubes #5, 6 and 7 you should do one at a time, i.e., add nothing to a tube until you are ready to follow the complete procedure. Starting with tube #5, add all ingredients asked for in the protocol except 1% urea, and let the tube rest for 2 minutes. Then add the 1% urea and start timing for the color change as you add it. Follow this procedure for the other two tubes. You should expect times of 2 minutes for #5, 10 minutes for #6, and 0.5 minutes for #7. The inhibition is competitive rather than noncompetitive because the tubes will eventually (about 10

minutes) turn pink.

Thus the active site is not permanently blocked.

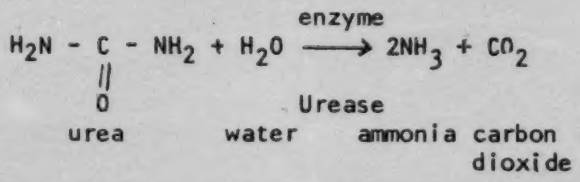
The crucial 3-D shape of urease is determined by hydrogen bonding, van der Waals forces and, most importantly, hydrophobic bonding. At temperatures between 60° and 80° C, hydrophobic bonding is greatly weakened because the increased thermal energy of the water prevents "ice sheath" formation around hydrocarbon residues. This allows the protein to unfold without unfavorable energy changes. Upon return to room temperature, thermodynamics favors refolding of the protein chain, but there are an enormous number of ways to fold which satisfy the thermodynamics. Consequently few, if any, enzyme molecules ever find their way back to the proper 3-D shape. This raises a very interesting point--how proteins manage to fold correctly in the first place, during their synthesis. Protein chemists are still arguing over this point.

Notes on Figure A.1 (Fumarase): This reaction was chosen for illustration because it is one of the simplest reactions catalyzed by an enzyme and therefore easiest to show schematically. The substrates fumaric acid and malic acid (for the reverse reaction) are given in the doubly charged anion forms (as these are the species present at the neutral pH where the enzyme functions).

mm test tube containing one milliliter of starch solution. After 10 minutes add 1 drop of iodine solution. Record any color that you observe. What are your conclusions?

A.3 PRELAB DISCUSSION

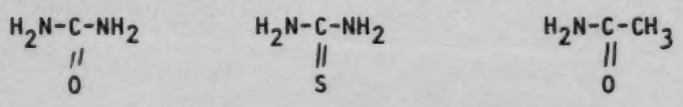
The enzyme urease catalyzes the hydrolysis of urea.



Make models!

The following will explore the question of how specific the enzyme urease is. Will urease catalyze the hydrolysis of molecules very similar in structure to urea?

Construct models of urea, thiourea and acetamide.



A.3 Experiment: SPECIFICITY OF UREASE

Obtain five milliliters of urease solution from your instructor. Add one drop of phenolphthalein indicator to each of the four 13x100 mm test tubes. Add one milliliter of 1% urea solution to each of test tubes 1 and 2, contain-

water and stir to form a paste. Add 95 ml of boiling water to the paste. Boil two minutes and use.

Discussion

1. Students will generally see no color after adding I_3^-. They will not usually see the red-brown of I_3^- because the solution is too dilute. They will not usually see the blue or purple of the starch -I_3^- complex because their spit will hydrolyze the starch. However, there is always the rare student who will not have ptyalin in his spit. This rare student should be encouraged to try the experiment on another day to see if his condition is permanent or temporary. The usual cause is gum chewing.

2. Without any delay to allow for enzymatic action the starch should be intact and therefore a blue or purple complex forms with the I_3^-.

3. Tenderizer will not catalyze the hydrolysis of starch. The blue starch - I_3^- complex will form.

4. Film: "Mechanism of an Organic Reaction" (CHEMS)

Materials and Equipment
(for one student)

7 test tubes
(13 x 100 mm)
3 droppers
Phenolphthalein indicator solution
Burner
1% urea (w/v) (3 ml)
Urease solution (5 ml)
4% thiourea (w/v) (2 ml)
4% acetamide (w/v) (2 ml)

Preparation of Urease Solution

Allow 10 grams of Jack Bean Meal (Mathison - Coleman - Bell) to soak in 500 ml of 30% (v/v) ethanol for at least ten minutes. Filter the solution and use the urease solution within one week if not refrigerated. Alternatively dissolve 0.1 g of urease in 500 ml of water.

Discussion

1. The pink color of basic (pH8-9) phenolphthalein will appear for any substance generating ammonia. Because urea, thiourea, and acetamide are so similar in structure, the concept of complementary surfaces on the enzyme urease should be strongly reinforced. Emphasize that the reaction is an hydrolysis and that the enzyme urease simply speeds the reaction.

2. The urease is quickly denatured by heating. Students have not encountered "denaturation" in this course. Discussion should be along the lines of destroying the complementary surface of the enzyme by breaking the weak bonds maintaining the shape of the surface. Mention the obvious physical and chemical differences between a raw and cooked egg.

3. Film: "Biochemistry and Molecular Structure" (CHEMS) (excellent) This film is a necessary introduction to an extension of the experiment covering competitive inhibition of

ing the indicator. To the third tube add one milliliter of 4% thiourea solution and to the fourth add one milliliter of 4% acetamide solution. Set aside test tube No. 1 containing urea. Add ten drops of urease solution to each of the three remaining test tubes. All of this information is summarized in the Urease Protocol on the following page. Record any color changes that occur before the end of the laboratory period.

Boil the remaining urease for ten minutes. After cooling the urease solution to room temperature, add ten drops of it to test tube No. 1, containing urea. Record the color change.

There seems to be a very sensitive relationship between the molecule hydrolyzed (urea) and the enzyme catalyzing the reaction (urease). Even the relatively mild exposure to a temperature of 100° destroyed the catalyzing ability of urease. The following discussion of polymer shape may help explain this sensitive relationship.

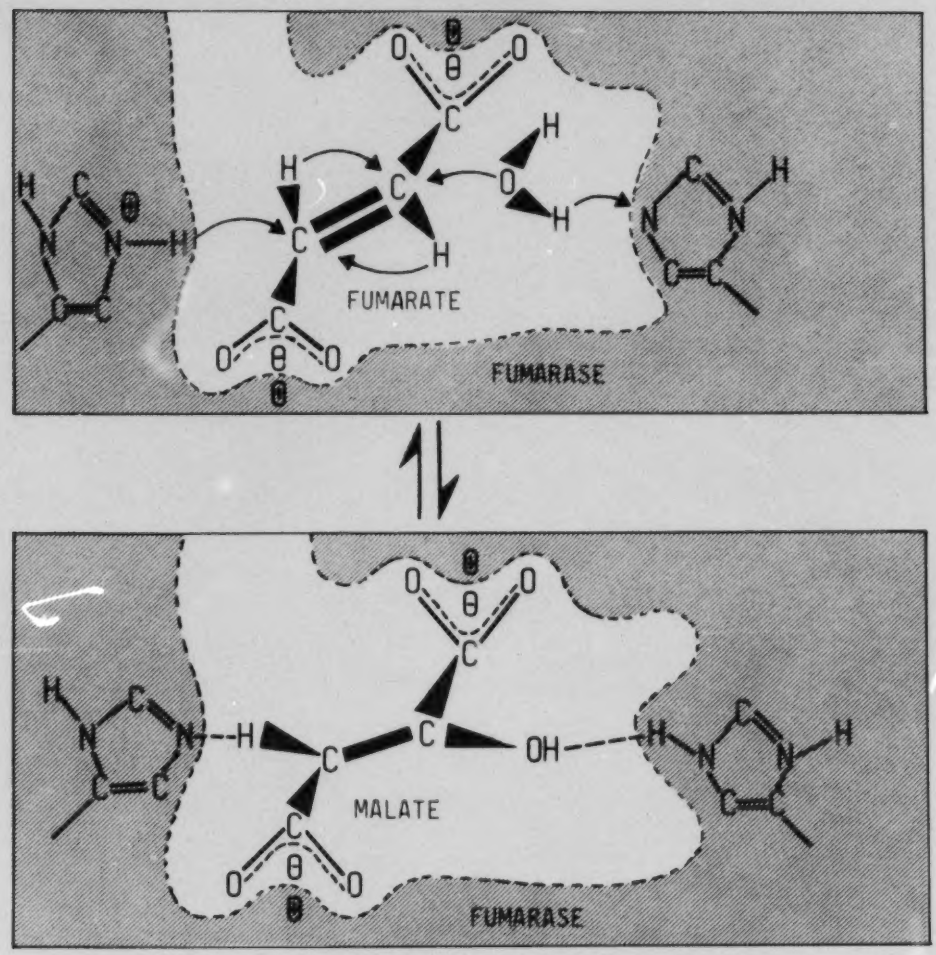
Supposing there is a complementary surface relationship between an enzyme and its substrate (the molecule undergoing reaction in the catalyzed reaction). Thus the enzyme is to the substrate what a lock is to a key. There are locks which operate with many keys and locks which are

UREASE PROTOCOL

reagent \ Test tube #	Specificity of urease				Substrate competition		
	1	2	3	4	5	6	7
Phenolphthalein	one drop	one drop	one drop	one drop	one drop	one drop	one drop
1% urea solution	1 ml	1 ml	—	—	4* drops	4* drops	4* drops
4% thiourea solution	—	—	1 ml	—	1 ml	—	—
4% acetamide solution	—	—	—	1 ml	—	1 ml	—
urease solution	—	10 drops	10 drops	10 drops	10 drops	10 drops	10 drops
boiled urease solution	10 drops	—	—	—	—	—	—
H_2O	—	—	—	—	—	—	1 ml

Table A.1

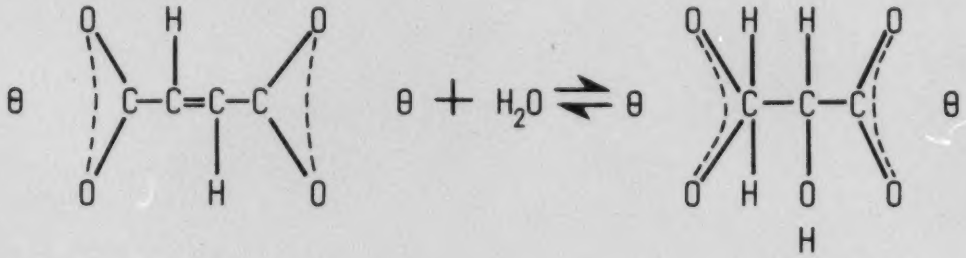
*Add this ingredient last: for each tube note and record the number of seconds elapsed from the time of this addition to the color change.



MECHANISM OF FUMARUSE ACTION

Figure A.1

The figure illustrates the reaction catalyzed by fumarase. It should give an idea of the active site of this enzyme and how the substrates fumarate and water fit into the active site, reacting to give malate:



(FUMARATE) MALATE

As indicated, the active site probably contains two small, positively-charged cavities at the proper locations to specifically bind fumarate into the active site. The imidazole rings (part of the amino acid histidine) to the right and left are the parts of the active site of the enzyme that catalyze the reaction.

specific for one key. There are enzymes which catalyze many substrates and enzymes which are specific for the catalysis of one substrate. (See Figure A.1)

Experiment A.3 showed that urease is specific for urea, even though the shapes of thiourea and acetamide are similar. This indicates that the fit between enzyme, urease and substrate, urea, is a close specific fit. Virtually every chemical reaction which occurs in living systems is catalyzed by a particular enzyme.

Information on the involvement of imidazole rings as well as other amino acid side chains in the catalytic process can be found in The Mechanism of Enzyme Action by S. A. Bernhard (Benjamin Co.), an excellent paperback on enzymes.

Exercises for Home, Desk and Lab (HDL's)

(1) What do you call enzymes which break down themselves as well as other proteins?

(1) Proteolytic enzymes

(2) Are all enzymes as specific in their behavior as was the enzyme urease on urea? Explain.

(2) No, all enzymes are not necessarily as specific as urease. There are some enzymes which catalyze many substrates.

(3) What would you hypothesize was the effect of the heat exposure of 100° C upon the enzyme urease?

(3) The effect of heat upon the enzyme urease was to destroy its catalysing ability. This may have resulted because the temperature increase was able to break up the hydrophobic bonding, which in turn destroyed the three-dimensional folding and thus the activity of the enzyme.

Since the temperature change is not very great, one should expect only the weakest type of bond to be broken in the enzyme. Once

the three-dimensional shape of a protein is destroyed, experiment has shown that the enzyme is not capable of reforming these weak bonds and resuming their activity.

(4) The ending of an enzyme is 'ase.'

(4) What ending is used to identify an enzyme when the name of the enzyme is written?

(5) Active part

(5) In a hydrolysis reaction does water act as a solvent only, a 'spectator' species only or does it play an active part?

(6) No. Saliva catalyzes starch to sugar. Urease catalyzes urea to NH_3 and CO_2. Hamburger contains protein, which is made up of amino acids.

(6) Do you think saliva would hydrolyze <u>hamburger</u>? Would urease? Why?

TEXT SECTION	ROUGH TIME ESTIMATES	EXPERIMENTS	DEMONSTRATIONS	TEACHING AIDS	OTHER STUDENT ACTIVITIES	PROBLEMS	OUTSIDE READING
Chap. VI Polymers to polymers	↑ 2 Days	A.1 Aspirin experiment		Note: Exp.A.1 Chap.1 Energy Capture and Growth requires that plants be started 2 weeks ahead		1, 5, 6, 7	
A. Activation	↓	A.2 The synthesis and breakdown of starch					
B. How cells make RNA C. How cells make proteins D. The master molecule E. Molecular symbiosis	↑ 3 Days ↓			Models Film: Gene action Film: DNA molecule of heredity	Models	2, 8, 9, 10 3, 4, 11 12, 13	Scientific American Oct 1962 Mar 1963 Oct 1966

Chapter VI: POLYMERS TO POLYMERS

Research on living things was greatly speeded up when it became obvious that all different kinds of organisms are fundamentally alike in their chemistry. For example the mechanism of protein synthesis in man is essentially the same as it is in plants or bacteria. By studying bacteria, which are easier to grow in the laboratory than plants or animals, it is possible to learn much that can be applied to more complex organisms.

NOTE: The first experiment in Chap. I, Energy Capture and Growth, requires that plants be started ahead. Plan a day two weeks ahead to plant beans according to the instructions in that lab.

The enzyme-catalyzed reactions which cells carry out can be divided into three main types. First, there are reactions which convert food molecules into the various monomers--the 20 amino acids, the sugars and the sub-units of the nucleic acids. Second, there are the reactions which convert either light or chemical bond energy into usable energy. This usable energy is then available to activate the nomomers. Third, there are the polymerization reactions: the activated monomers are linked together in the proper sequences to produce the polymers which make up the cell. A great deal is already known about the first topic, the breakdown of food

into small molecules. Less is known about the polymerization reactions. Much remains to be discovered about the formation of usable energy.

A. THE ACTIVATION PROBLEM

In order to make the monomers of nylon polymerize it was necessary to change one of them to a high energy form. The adipic acid was converted to an activated form (adipoyl chloride) which reacted spontaneously with hexamethylene diamine to give nylon and HCl.

This is the reaction: nylon → monomers. Δ H is negative and Δ S is positive, or:

$\Delta G = \Delta H - T\Delta S < 0$

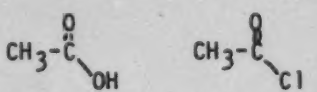
Nylon can be considered to have more chemical energy than an equivalent solution of its monomers. As a consequence nylon will react spontaneously with H_2O to yield the monomers. This reaction, however, is extremely slow in the absence of a catalyst.

Aspirin Synthesis Experiment
(equipment for one student)

- *6 test tubes (13x100 mm)*
- *1 bunsen burner with ring stand and wire gauze*
- *1 250 ml beaker*
- *1% (w/v) salicylic acid solution in 1, 4-dioxane (5 ml)*
- *1% (w/v) acetyl-salicylate solution in 1, 4-dioxane (1 ml)*
- *0.1% (w/v) $FeCl_3$ solution in H_2O (6 ml)*

Ventilating hood; three teflon-stopcock burets containing acetyl chloride, acetic anhydride and glacial acetic acid respectively. Also, eye-

Activation of one of the reactants is often required to make a reaction "go." When one of the reactants is a carboxylic acid the usual activated form in the laboratory is an acyl chloride; e.g.

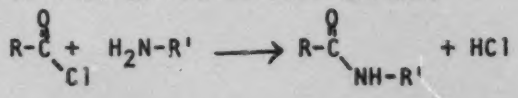


acetic acid acetyl chloride (an acyl chloride)

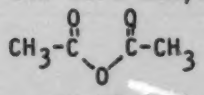
This functional group of an acyl chloride ( ) contains more chemical energy than the carboxyl group. As a result acyl chlorides will

react vigorously with alcohols to form esters:

or with amines to form amides:



Another activated form of carboxylic acids is the acid anhydride:



acetic anhydride

droppers at some central location provide an easy way to dispense the salicylic acid, acetyl salicylic acid and $FeCl_3$ solutions.

Warning: Acetyl chloride, glacial acetic acid and acetic anhydride can cause burns. If eye-droppers are used instead of burets, it is almost certain that a student will be injured.

A.1 Experiment: ASPIRIN SYNTHESIS

Aspirin Synthesis Protocol						
Reagent	Tube Number 1	2	3	4	5	6
Salicylic acid 10 mg/ml in dioxane	1 ml	—	1 ml	1 ml	1 ml	1 ml
Acetyl salicylic acid 10 mg/ml in dioxane	—	1 ml	—	—	—	—
Acetyl chloride	—	—	0.5ml	—	—	—
Acetic anhydride	—	—	—	0.5ml	1 ml	—
Glacial acetic acid	—	—	—	—	—	1 ml
Heat for ten minutes in boiling water bath before adding $FeCl_3$						
$FeCl_3$, 1 mg/ml in water	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml

Table A.1

See: H.O.J. Collier, "Aspirin", Scientific American, Nov. 1963, Reprint No. 169

No. 1, 4 and 6. The color in 4 may be noticeably less intense.

Salicylic acid and Fe^{+3} react to form an intensely purple chelate.

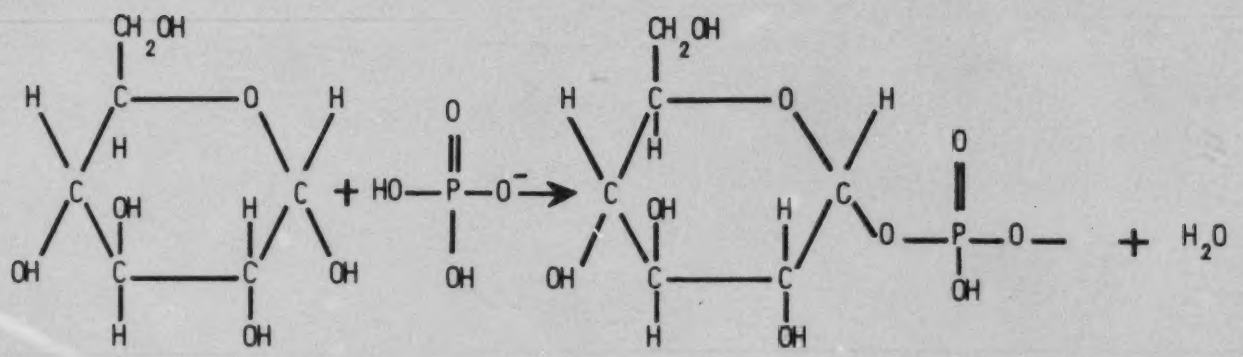
Tubes 3 and 5 with some synthesis probable in tube 4.

We will now use salicylic acid with acetyl chloride and acetic anhydride to make an ester, acetyl salicylic acid, which is usually called aspirin. In which tubes did you obtain a purple color? What combination of compounds is responsible for the purple color? In which tubes was aspirin (acetyl salicylic acid) formed?

Cells produce polymers, such as starch proteins and nucleic acids which also contain more chemical energy than their monomers. Therefore, in order to form polymers cells must convert monomers into higher energy derivatives which are called activated monomers.

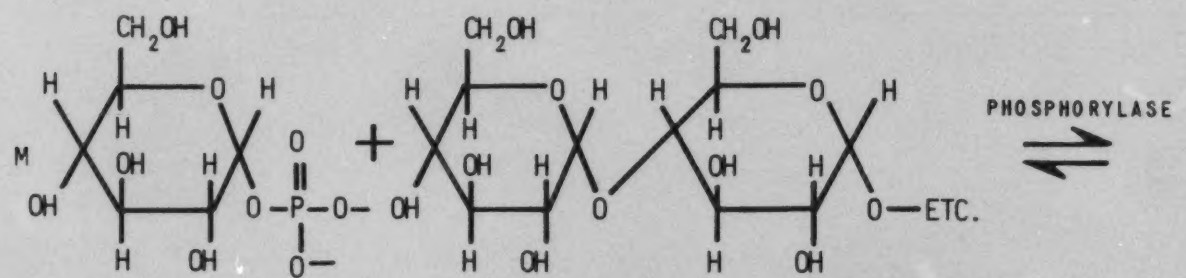
Why or how living systems came to use phosphoric acid anhydrides and esters for activation is unknown. But one thing is clear. Unlike acyl chlorides such phosphates are quite stable in water at neutral pH in spite of their high energy content. This means that they will react only via enzymic catalysis, which is of course an indispensable prerequisite for a cell.

Cells activate monomers in quite different ways than does the organic chemist. One way is to attach a phosphate group to the molecule to be polymerized. For example, glucose can be made to polymerize to starch if it is first converted to glucose-1-phosphate. Glucose-1-phosphate reacts reversibly with a starch molecule to form a phosphate ion and a starch molecule with one more glucose residue. The reaction is catalyzed



GLUCOSE PHOSPHATE GLUCOSE-1-PHOSPHATE (IONIZED FORM)

by an enzyme called phosphorylase and can be written as follows:



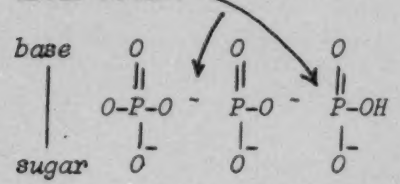
GLUCOSE-1-PHOSPHATE PRIMER OR GLUCOSE$_n$

STARCH OR GLUCOSE $(n+m)$ HYDROGEN PHOSPHATE ION

This reaction of activated monomers is readily reversible: a glucose subunit can either be added to or removed from the end of a starch molecule. Whether the reaction goes in the direction of elongation or breakdown depends mainly on the relative concentration of glucose-1-

$$K_{eq} = \frac{(\text{STARCH})\ (HPO_4^{-2})}{(\text{GLUCOSE-1-PO}_4)\ (\text{PRIMER})}$$

ATP has two phosphoric anhydride bonds.



For more detailed information about ATP and its role in energy transfer, the teacher is referred to Watson, The Molecular Biology of the Gene, Benjamin, 1965, Chapter 5; McElroy and Swanson, Modern Cell Biology, Foundations of Biology Program, Part 2, Prentice-Hall, Chapter 10; Lehninger, A., Bioenergetics, Benjamin, 1965, Chapter 4.

Have a demonstration model of ATP built. You could request that the class build this using FMM kits. Make phosphorus tetrahedral (sp^3).

Materials and Equipment (for one student)

4 test tubes (100x13mm)
1 spot plate, if available
1 beaker
1 universal indicator paper

Solutions: (for class)

(1) substrate solution I

glucose-1-phosphate	*0.2 g*
glycogen [primer]	*0.2 g*
water	*50 ml*

(2) substrate solution II

glucose	*0.2 g*
glycogen [primer]	*0.2 g*
water	*50 ml*

Dissolve the glucose-1-phosphate or glucose in 20 ml water. Using universal indicator paper, adjust the pH

phosphate and phosphate ion. If glucose-1-phosphate is high and phosphate ion is low, starch is elongated; if phosphate ion is high and glucose-1-phosphate is low, then starch is broken down. How do cells activate glucose to glucose-1-phosphate? The answer is that they use a high energy organic phosphate compound whose name is usually abbreviated to ATP (adenosine triphosphate). ATP is at a higher energy level so it can easily donate one of its phosphate groups to glucose. A large part of the metabolism of cells is devoted to the formation of ATP. This was referred to earlier as usable energy.

A.2 Experiment: THE SYNTHESIS AND BREAKDOWN OF STARCH BY PHOSPHORYLASE

Place one ml of phosphorylase solution in a test tube labeled B (for boiled) and three ml of phosphorylase solution in a test tube labeled C (for cold). Place tube B in boiling water for ten minutes.

Add 1.0 ml of solution containing glucose-1-phosphate and primer (a starch-like polymer which is too short to give a blue color with iodine) to each of four 13 x 100 mm test tubes. To a fifth tube add 1 ml of glucose and primer solution. The numbering of the tubes is given in the Phosphorylase Protocol (page 113). To the first tube add one ml of boiled phosphorylase

solution B. To the second, third and fifth tubes add one ml of unboiled phosphorylase solution C. Add one ml of distilled water to the fourth tube. At zero time and at three minute intervals thereafter, test each reaction mixture for starch synthesis by adding a drop of iodine solution to a drop of reaction mixture on a spot plate. A blue color indicates the presence of starch. As soon as you have evidence of starch synthesis, carry out the following steps. First, add 1 ml of potassium phosphate solution to all test tubes and continue to test for starch. Next, inactivate the enzyme in tube 2 by placing the tube in a boiling water bath for 10 minutes. How do you explain the disappearance of starch in tube 3 but not in tube 2? Did you expect any starch to be formed in tube 5?

to 6.1-6.3 with HCl and dilute to 25 ml with water.

Dissolve the glycogen in 25 ml water. Boil, filter and mix the glycogen solution with the glucose-1-phosphate (or glucose) solution.

(3) phosphorylase solution

Peel a small potato and cut it into small cubes. Place these in a blender, add 40 ml of 0.01M sodium fluoride and homogenize for 30 seconds. The purpose of the sodium fluoride is to inhibit the potato enzyme phosphatase, which would otherwise hasten the hydrolysis of glucose-1-phosphate to glucose and phosphate.

Filter the homogenate through a double layer of cheesecloth into a beaker. Squeeze out as much of the liquid as you can. Centrifuge the suspension for 3 minutes, then decant and keep the supernatant. Take a small portion of this extract and see that it tests negative to the iodine test for starch. This should be enough enzyme for the whole class and should retain its activity for nearly a week if carefully refrigerated. The enzyme preparation is used full strength in the experiment.

Because potatoes do contain some short-chain starch (primer), it is not absolutely necessary to add primer in the experiment. However, if glycogen (primer) is added, then starch will appear in less than five minutes. If glycogen (primer) is not added, then it will be about fifteen minutes before starch

appears. Thus if lab time is short in your school, add primer to the glucose-1-phosphate solution. If there is ample lab time in your school, omit primer from the glucose-1-phosphate solution.

(4) potassium phosphate

Make 50 ml of 1 M potassium or sodium dihydrogenphosphate (KH_2PO_4 or NaH_2PO_4). Add 13.9 ml of 1 M sodium hydroxide. Dilute the mixture to 100 ml with water.

(5) iodine solution

Dissolve 1.5 g KI in 25 ml water. Add 0.3 g I_2; stir until dissolved. Dilute to 150 ml with water.

Starch disappeared from tube 3 because the enzyme phosphorylase catalyzed the phosphorolysis of starch by the excess phosphate ion. This is a simple example of LeChatelier's Principle.

$$\text{starch} + P_i \xrightleftharpoons{\text{phosphorylase}} \text{glucose-1-P}$$

Tube 2 did not catalyze this reaction because the enzyme phosphorylase had been inactivated by heat.

The starch-I_3^- blue color is due to a clathrate complex (cage complex) in which a section of the helical starch molecule "wraps" around a string of iodine atoms. There is a minimum required length for the starch helix to give the blue color. A shorter length gives a red or purple color. Shorter lengths than this give no color.

PHOSPHORYLASE PROTOCOL

Reagent \ Test Tube #	1	2	3	4	5
glucose-1-phosphate and primer solution	1 ml	1 ml	1 ml	1 ml	____
glucose and primer solution	____	____	____	____	1 ml
boiled phosphorylase (B)	1 ml	____	____	____	____
phosphorylase (C)	____	1 ml	1 ml	____	1 ml
Distilled H_2O	____	____	____	1 ml	____
Test plate iodine solution	1 drop	1 drop	1 drop	1 drop	1 drop
Color observed					

Table A.2

B. THE SEQUENCE PROBLEM OR HOW CELLS MAKE RNA (RIBONUCLEIC ACID)

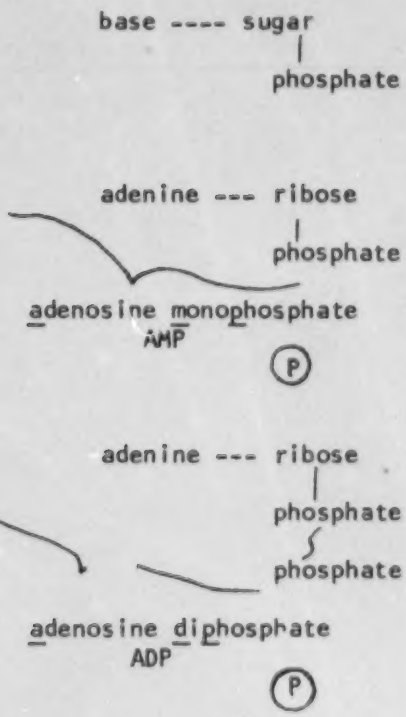
In Chapter II you saw that RNA is made of four different kinds of monomers linked together to form long chains. The monomers (residues) found in RNA include the bases adenine, guanine, cytosine and uracil (abbreviated A, G, C and U).

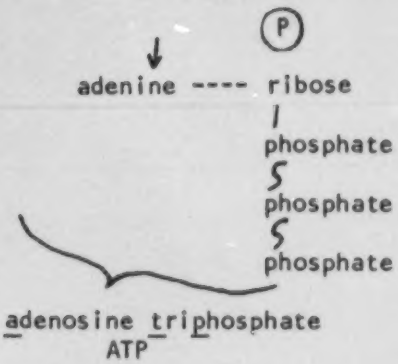
Remember that the monomer is the small molecule by itself and the residue is what is left of this when it is in the polymer. The structures of these four bases as well as that of thymine, found in DNA, are shown in Figure B.1 of Chapter IV.

RNA molecules vary from 70 residues to over 10,000 residues in length. Since each sequence of monomers gives a different RNA molecule, the number of possible RNA molecules is so vast as to be beyond comprehension. For example, there are four possible choices (bases) for each position in the sequence of an RNA molecule: For molecules four bases long there are, therefore, $(4)^4$ or 256 possible different sequences. How many different sequences are possible for RNA molecules 10 bases long? This means that if the monomers of RNA were polymerized at random no two molecules of RNA could be expected to be alike. However cells do not produce random RNA molecules; rather they produce several thousand

specific types of RNA molecules. Each kind of cell has its characteristic set of RNA molecules and it can produce many copies of each kind.

Thus the cell is faced with two problems in synthesizing RNA. It must activate the monomers of RNA so that polymerization will be favored, and it must direct the sequence of polymerization so that the correct RNA molecules are formed. The first task requires ATP, which donates phosphate groups to the other monomers to form triphosphates. ATP is itself an activated monomer of RNA, but its formation is an extremely complex process.





adenosine triphosphate
ATP

The activation of the other monomers occurs by means of the following types of reactions. We will use the activation of uridine monophosphate (UMP) as an example.

1. $UMP + ATP \overset{\text{enzyme}}{\rightleftharpoons} UDP + ADP$
2. $UDP + ATP \overset{\text{enzyme}}{\rightleftharpoons} UTP + ADP$

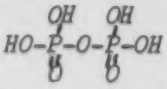
Here U stands for the base-ribose combination, uracil-ribose, called uridine. Thus the activation of UMP requires the "expenditure" of two ATP's. These ATP's are replenished by means of the energy-capturing reactions of the cell.

See Part IV, Chapter I for a presentation of energy capture to form ATP.

The second task requires a molecule which will align the monomers in proper sequence for polymerization. This molecule is DNA. A common term for this type of alignment device is template; DNA is a template molecule.

The polymerization reaction may be expressed as follows:

Dihydrogen pyrophosphate is



formed by buffering action of cell medium.

$$n \begin{Bmatrix} ATP \\ GTP \\ CTP \\ UTP \end{Bmatrix} \xrightarrow[\text{RNA polymerase}]{\text{DNA (template)}} \text{RNA} + 4n(P\text{-}P)$$

Uracil and thymine have the same base pairing properties.

where P-P stands for the inorganic ion, pyrophosphate. The RNA formed has the same base sequence as one of the DNA strands except that the base uracil is substituted for the base thymine of DNA. Precisely how the enzyme RNA polymerase and template DNA cooperate to specify the RNA base sequence is not known. Almost certainly hydrogen bond base pairing between the incoming activated monomers and the template DNA strand is an essential factor.

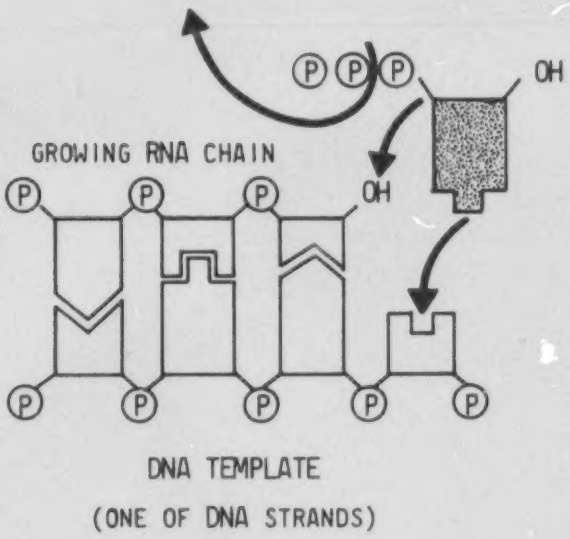


Figure B.1

C. THE SEQUENCE PROBLEM CONTINUED--HOW CELLS MAKE PROTEINS

The character and individuality of cells arise from the protein molecules they are able to synthesize. As enzymes these molecules determine the pattern of reactions the cell can carry out as well as the shape and size of cells (structural proteins and regulatory proteins). As you have seen, protein molecules can assume great varieties of shapes, depending primarily on their sequence of amino acids. Because of this, life itself has enormous diversity, versatility and adaptability. Proteins are the vehicles of expression of the hereditary traits.

Refer to Chapter III for molecular shape.

For clear useful color diagrams of both protein synthesis and genetic processes see:
Time Magazine
April 19, 1971
section on genetics

As in the case of RNA the number of different protein molecules possible is enormous, yet a given cell makes only a limited set of protein molecules. Again as in the case of RNA, the cell must both activate the amino acid monomers and polymerize the activated amino acids in proper sequence. This turns out to be a more complex process than the production of RNA.

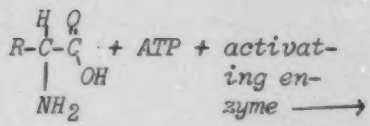
The amino acids are activated by attachment to a special type of RNA called transfer RNA or tRNA for short. There are about 40 different tRNA molecules, each about 70 residues in length. Each tRNA can attach to one and only one amino acid. (Since there are only 20 different amino

The attachment of an amino acid to its transfer RNA goes in two steps:

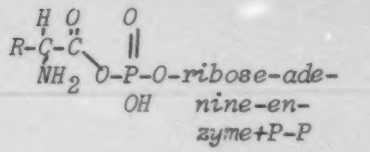
(1) Amino acid+ATP+ activating enzyme ⟶

amino acid-AMP-enzyme+ P-P

or



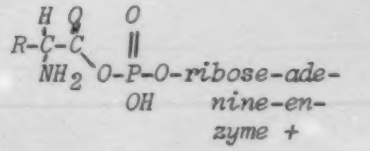
+ ATP + activating enzyme ⟶



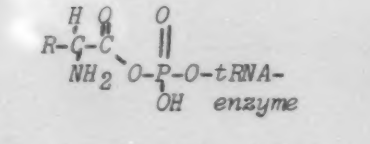
(2) Amino acid-AMP-enzyme+ tRNA ⟶

amino acid-tRNA-enzyme+AMP

or



tRNA ⟶ AMP +



Note activation of amino acid by anhydride formation.

acids in proteins, some amino acids must have more than one corresponding tRNA.) The linking of a given amino acid to its tRNA requires ATP and a special enzyme.

The sequence of amino acids in a protein is determined by the sequence of bases in an RNA template called messenger RNA or mRNA. You will remember that a DNA strand acts as a template for RNA by forming hydrogen bonds with the activated RNA monomers. However amino acids by themselves can't hydrogen-bond to DNA or RNA. Therefore, in order for mRNA to act as a template for amino acid polymerization it is necessary for the amino acids to be linked to adaptor molecules that <u>can</u> hydrogen-bond to the bases of the mRNA. This is precisely what the tRNA molecules do. When tRNA molecules linked to their amino acids align themselves on mRNA, the amino acids are brought close together, and since they are in an activated state they can polymerize (in the presence of an enzyme). Thus the tRNA has two roles: it is an activator and it enables the base sequence of an mRNA molecule to be "translated" into the amino acid sequence of a protein molecule.

What are the rules for this translation - in other words, what is the "code" by which the

"sentences" of mRNA are translated into "sentences" embodied in the structure of proteins? In the past 10 years a great deal of work has been done on the coding problem, using isolated protein-synthesizing systems from many types of cells. It has been possible to use chemically synthesized mRNA of known base sequence to direct amino acid polymerization and then to analyze the protein formed for its amino acid sequence.

The genetic code is now completely cracked. It is a triplet code--that is, it takes a sequence of three consecutive nucleotides in the mRNA to specify one amino acid. In protein synthesis the mRNA is "read" three nucleotides at a time, starting from one end of the molecule and proceeding to the other without skips. Each triplet of nucleotides is called a codon. There are 64 possible codons--that is, there are 4^3 (64) possible sequences of A, G, C and U taken three at a time. There are only 20 amino acids in proteins, and it turns out that several codons can specify the same amino acid. For example, the amino acid glycine is specified by any one of the following codons: GCU, GCC, GCA, GCG. All but three of the 64 codons have been assigned to a specific amino acid. The remaining three

The following are excellent references to the genetic code.

Cold Spring Harbor Symposium on Quantitative Biology, 1966, Vol. 31.

Scientific American
October 1962
March 1963
October 1966

codons are probably used to end a protein molecule; that is, they serve the same purpose as a period does for a sentence.

Since each amino acid to be polymerized is aligned on the mRNA, it follows that each kind of tRNA must have a specific "anti-codon"--a sequence of bases which can hydrogen-bond to the codon of the mRNA. For example, if the codon on the mRNA is UUU then the anticodon on the tRNA is AAA.

The mRNA and the various tRNA-amino acid combinations are brought together in or on the ribosomes. Ribosomes are particles found in the cytoplasm of cells. (See Figure C.2.) They are composed of RNA and protein, and it is here that the actual polymerization of amino acids takes place. The process of protein synthesis can be diagrammed as in Figure C.1.

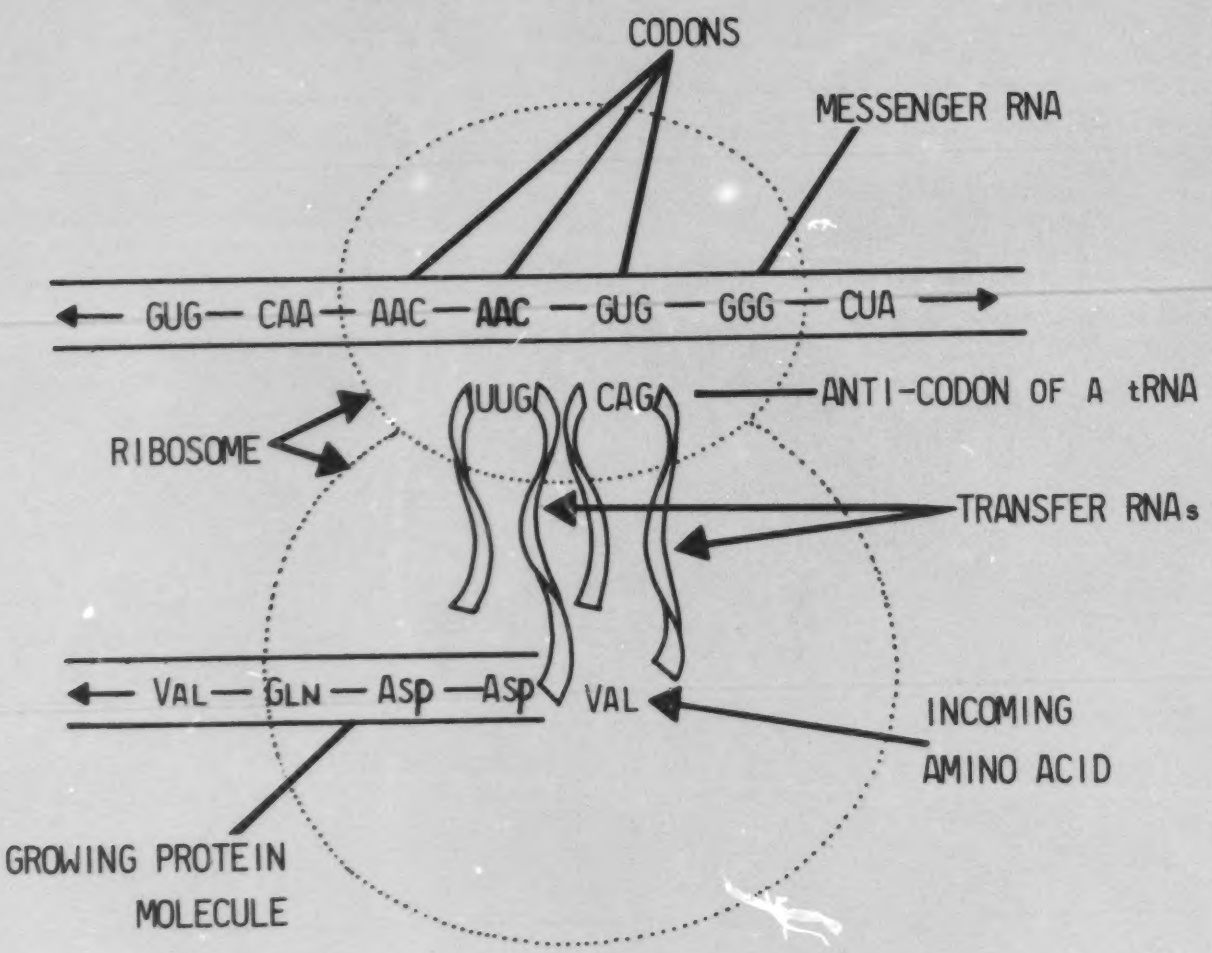

CODONS
MESSENGER RNA
GUG — CAA — AAC — AAC — GUG — GGG — CUA
UUG
CAG
ANTI-CODON OF A tRNA
RIBOSOME
TRANSFER RNAs
VAL — GLN — ASP — ASP
VAL
INCOMING
AMINO ACID
GROWING PROTEIN
MOLECULE

Figure C.1

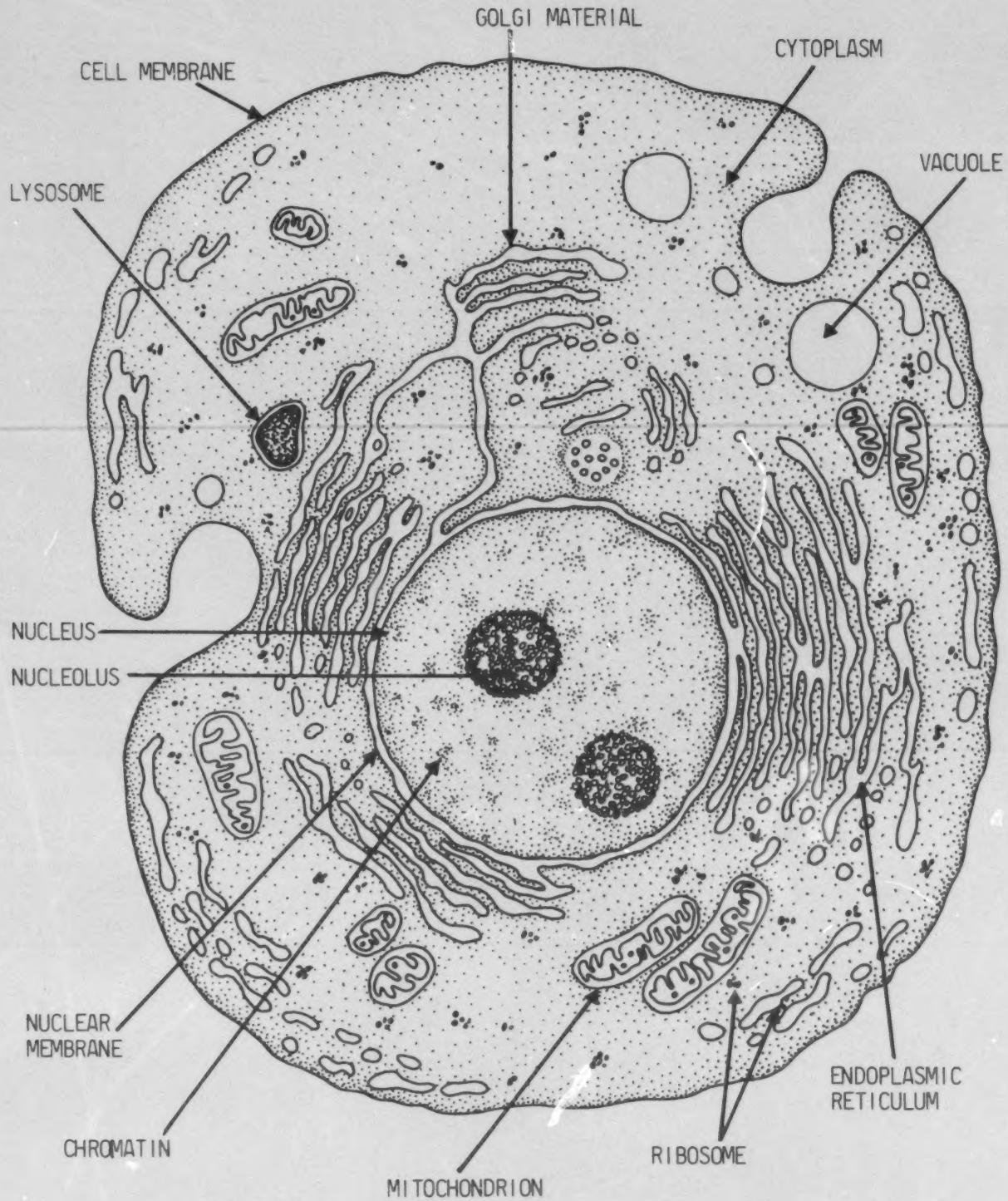


Figure C.2 An Idealized Animal Cell

D. THE SEQUENCE PROBLEM COMPLETED OR THE MASTER MOLECULE

See again: Time magazine. April 19, 1971.

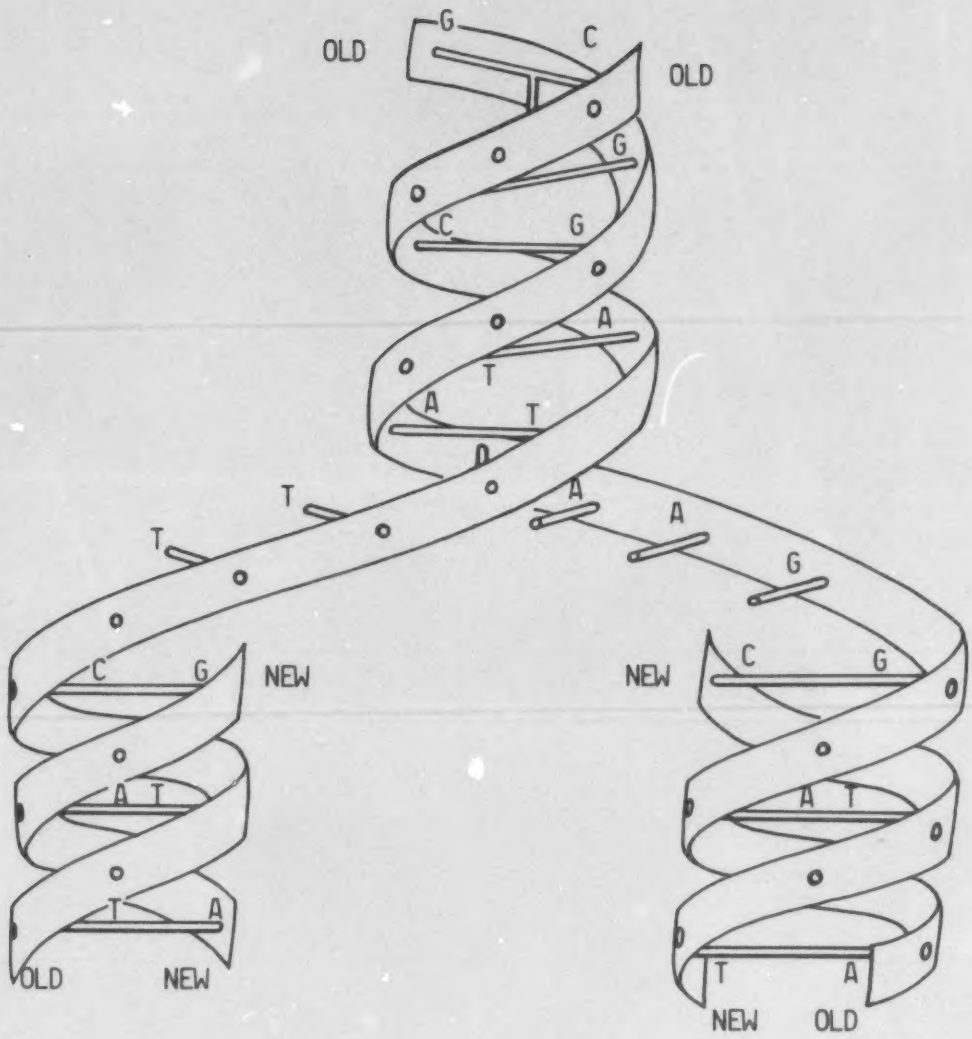
You have seen that the polymerization of amino acids is not a random polymerization, because the insertion of amino acid subunits is directed by the sequences of bases in mRNA. The polymerization of RNA, in turn, is controlled by the sequence of base pairs in DNA. Thus the structures of all proteins in the cell are controlled indirectly by the base pair sequence of DNA.

Actually DNA only contains one type of instruction or information--namely its sequence. This sequence is replicated into an RNA molecule by one enzyme and into a new DNA molecule by another enzyme. Note: for RNA transcription only one of the two DNA strands is read, while both are read in making a DNA copy.

When DNA is synthesized, where are the instructions for its polymerization? The answer is: in the pre-existing DNA molecule. Thus DNA combines two types of instructions in its base sequence--one for RNA and one for its own replication. It is this dual role of DNA which makes it unique--the master molecule. Because of the specific pairing that exists between the bases, the two strands of DNA are complements of each other. This means that each strand of the double helix can act as a template for the synthesis of the other.

As in the case of RNA synthesis, DNA synthesis requires activation of monomers to the triphosphate level and the presence of a specific enzyme or enzymes. One of the enzymes involved in DNA replication, called DNA polymerase, has

been obtained in pure form and used to study DNA replication in the test tube. Not all aspects of DNA replication have been worked out, but it is known that replication starts at one end of the molecule and proceeds to the other and that each of the two daughter molecules consists of one old strand and one newly-synthesized strand. This is what would be expected if replication occurs as diagrammed on the following page.



DNA REPLICATION

Figure D.1

It turns out that DNA molecules are the longest of the biopolymers. Probably each chromosome of the cell contains only one huge molecule of DNA. In the case of human chromosomes this means that single DNA molecules would be as much as 7.5 cm in length. Each such DNA molecule could code for up to 100,000 different proteins.

7.5 cm of DNA would be around 2 x 10^8 base pairs in length. Assuming that most protein chains are 500 amino acids in length, the figure of 100,000 different proteins is obtained.

E. MOLECULAR SYMBIOSIS

The polymers of the cell interact in a way that might be described as symbiosis on the molecular level. Each kind of polymer contributes to the synthesis of the other kinds. DNA acts as a storehouse of information for the synthesis of the thousands of protein molecules of the cell; RNA serves as a part of the translation machinery; and the proteins themselves are the universal catalysts and building blocks of the cell. Without proteins, nucleic acids could not be synthesized.

Symbiosis is biological cooperation between two different kinds of organisms.

Exercises for Home, Desk and Lab (HDL's)

(1) In the polymerization of glucose to starch, the glucose first must be activated. What is the activated form of glucose?

(2) In the formation of RNA what are the activated monomers?

(3) When amino acids are polymerized to proteins, how are the amino acid monomers activated?

(4) Itemize the reagents needed by a cell to produce protein containing ten different amino acids.

(5) What are the general types of reactions which are catalyzed by enzymes in cells?

(6) a. What are two activated forms of acetic acid?

b. What is the structure of these two activated forms?

c. Write out two reactions which may occur with one of the activated forms.

(1) Glucose - 1 - phosphate

(2) ATP GTP CTP UTP

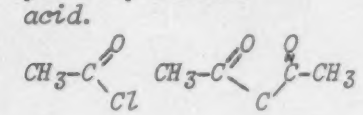
(3) With transfer RNA (tRNA)

(4) Ten amino acids
1 tRNA for each amino acid (10)
1 ATP to attach each tRNA to its amino acid (10)
1 enzyme to catalyze each tRNA-amino acid attachment
1 mRNA for template
Enzymes to polymerize the activated amino acids on template in RNA
If we go back a step and make the DNA from which the RNA is formed, the list becomes enormous.

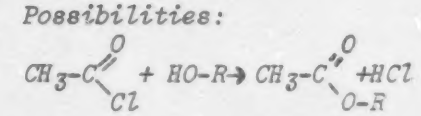
(5) a. Foods (polymers) are converted into monomers (amino acids).
b. Chemical bond energy is used to activate monomer.
c. Polymerization reactions, linking the activated monomers in the proper sequence.

(6) a. Acetyl chloride and acetic anhydride are two activated forms of acetic acid.

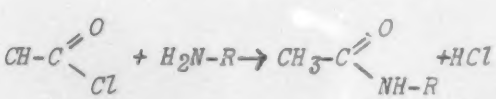
b.



c. Possibilities:



an ester



an amide

(7) a. phosphorylase

b. The starch polymer is elongated if glucose - 1 - phosphate ion concentration is high and phosphate ion concentration is low.

c. If the phosphate ion concentration is high and glucose - 1 - phosphate is low, then starch is broken down.

(7) Cells may activate the monomer glucose by adding a phosphate group.

a. What enzyme polymerizes the activated monomer into the $glucose_n$ primer?

b. Under what conditions does the reaction proceed to elongate the primer?

c. Under what condition does the reaction proceed to shorten the elongated polymer?

(8) The cell must activate the monomers of RNA so that polymerization will be favored, and it must direct the sequence of polymerization so that the correct RNA molecules are formed.

(8) What are two problems a cell is faced with in synthesizing RNA?

(9) DNA

(9) What is the template molecule for RNA?

(10) RNA differs from DNA in that the pyrimidine, uracil, has replaced the pyrimidine thymine. In addition, RNA is based upon ribose (a pentose sugar) while DNA is based upon desoxyribose.

(10) In what way does RNA differ from DNA?

(11) The sequence of amino acids in a protein is determined by the sequence of bases in an RNA template called messenger RNA, labelled mRNA.

(11) How is the sequence of amino acids in a protein determined?

(12) DNA combines two types of instructions in its base sequence--one for RNA and thus proteins and one for its own replication.

(12) DNA in its unique role as a master molecule carries two types of instructions for the molecules of a cell. What are they?

(13) In or on ribosomes.

(13) Where does the cell manufacture proteins?

TEXT SECTION	ROUGH TIME ESTI-MATES	EXPERIMENTS	DEMONSTRATIONS	TEACHING AIDS	OTHER STUDENT ACTIVITIES	PROBLEMS	OUTSIDE READING
Chap. VII Genes, proteins and mutations	2 Days			"PKU Detection" available from Oregon State Dept. of Health Prepare agar for Exp. C.1 2 or 3 days in advance		1-7	

Chapter VII: GENES, PROTEINS AND MUTATIONS

A. MISTAKES IN GENETIC MATERIAL

In order for cell duplication to be precise, it is necessary that the genetic material (DNA) be replicated exactly and distributed to the daughter cells. The fidelity of DNA replication is extremely high, but it is not perfect. There is a small but significant chance of mistakes whenever a DNA molecule is replicated. Mistakes can be of several kinds. The simplest and most frequent is the random insertion of the wrong monomer, leading ultimately to the change of a base pair. Other kinds of accidents may involve addition or deletion of base pairs or other gross rearrangements of the DNA base sequence. Often such errors are caused by radiation or by certain chemicals. Whatever the nature of the accident, once made it is replicated with the same high fidelity as the original sequence.

A base change mutation can come about as follows:

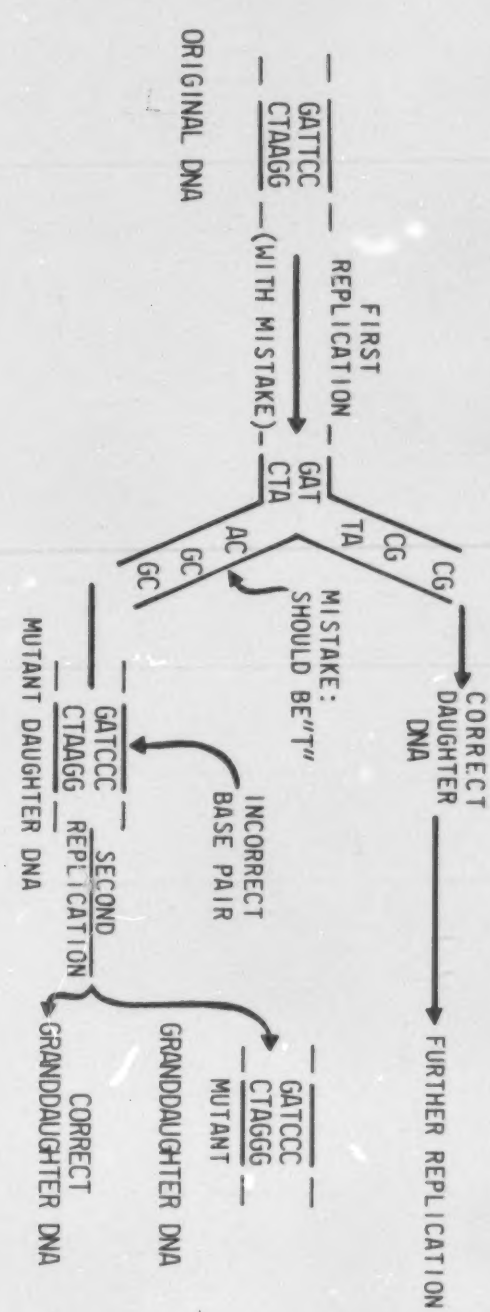


Figure A.1

Two points: 1. Mutation can show up (be expressed in the phenotype) after either the first or second round of replication. Remember that only one strand of the DNA is used as a template for mRNA synthesis. We will call this the "sense" strand and the complementary strand the "anti-sense" strand. If the incorrect base is put into the "sense" strand, immediately all mRNA and protein derived therefrom are altered. If the mutation is in the "anti-sense" strand, obviously a "sense" strand (with the mistake) must be copied before the mutation is expressed.

2. Only one of the four granddaughter DNA molecules is mutant.

Examples of mistakes where protein alterations do not occur are:

1. a base pair change of mRNA codon to a synonym, e.g. GCU to GCC, both of which designate glycine; or

2. a base pair change in a segment of the DNA which codes for ribosomal RNA, i.e., does not specify a protein.

Show the PKU film "PKU Detection," available from the Oregon State Dept. of Health.

A prepared slice showing sickle cells can be obtained from Carolina Biological Supply, Cat. #PH 1015 Sickle Cell Anemia - $1.25 per slide. The base pair change would be from A- T to T - A corresponding to an mRNA codon change from GAA to GUA.

This ability of the genetic material to perpetuate sequence changes is a very important property of living things. It is the source of the enormous diversity found in the biological realm. Any inherited alteration of DNA is called a mutation. Many of these change the sequence of an mRNA molecule and thus the sequence of a protein molecule.

B. EFFECTS OF MISTAKES

A change of even one amino acid in the amino acid sequence of a protein can have profound effects. A classical example of this is provided by sickle cell anemia. Certain people carry a mutant gene controlling the amino acid sequence of part of the hemoglobin molecule. The mutant hemoglobin sequence differs from normal hemoglobin by only one amino acid out of a total of about 140. Position six of the mutant hemoglboin is occupied by an amino acid called valine, while normal hemoglobin has an amino acid called glutamic acid at this position. In all other respects the two sequences are identical. Yet because of this single amino acid difference the mutant hemoglobin is less soluble than normal hemoglobin. It tends to crystallize within the red blood cells, especially when the oxygen concentration is low. The hemoglobin

crystals distort the red cells into odd crescent shapes--hence the name sickle cell. The sickle cells are rapidly destroyed by the body, apparently because they are damaged. For this reason the afflicted individual develops severe anemia and usually dies by the age of two.

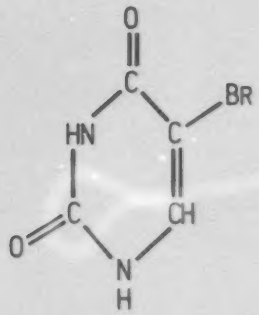
This whole train of events is probably the result of the change of a single base pair in the DNA of the hemoglobin gene. There are many examples in which the change of a single amino acid in the structure of an enzyme strongly affects the catalytic activity of the enzyme. The most frequent effects are complete loss or drastic reduction in catalytic activity. This should not be surprising, since natural selection has been refining the catalytic efficiency of most enzymes for many millions of years.

C. CAUSING MISTAKES

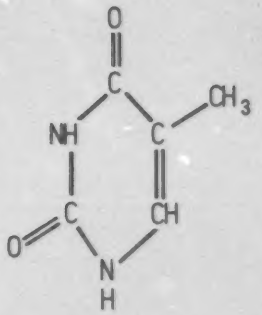
If some mutations are the result of base pair changes causing amino acid changes, it should be possible to find chemicals which increase the possibility of mistakes in DNA replication and which therefore increase the frequency of mutation. One such chemical is bromouracil (BU). This compound has a structure which is identical to thymine except that a bromine atom is substituted for the methyl group of thymine.

	Normal	*Sickle*
DNA ...	*GAA* ...	... *GTA*
	GTT	*GAT*
mRNA...	*GAA* ...	... *GUA*
hemoglobin ...	*glu* ...	... *val*

This is true only for individuals homozygous for sickle-cell anemia. People heterozygous for this trait, i.e. carrying one normal and one mutant gene, usually have a normal lifespan, although they can be adversely affected by violent exercise or high altitudes.



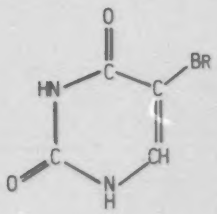
BROMOURACIL



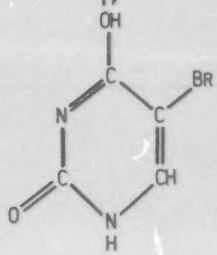
THYMINE = METHYLURACIL

BU Tautomers

BU exists as an equilibrium mixture of two tautomeric forms--the keto and the enol tautomers. The keto form is the predominant one.



KETO FORM PAIRS WITH ADENINE



ENOL FORM PAIRS WITH GUANINE

Errors can occur at the time BU is first incorporated into DNA if BU mistakenly replaces cytosine. Errors can also occur during subsequent replications when BU directs the insertion of guanine instead of adenine in the new DNA strand.

Bromouracil is able to "fool" DNA polymerase. Cells grown in the presence of BU incorporate it into their DNA. However the pairing properties of BU are not as precise as those of thymine: BU usually pairs with adenine, but sometimes it exists in a form which pairs with guanine. Because of this imprecise pairing behavior, DNA containing BU is much more likely to make mistakes during replication. Therefore, BU greatly increases the frequency of mutations.

Each gene (section of DNA which specifies a protein) has a characteristic probability of detectable error during each replication. For most genes this spontaneous mutation rate is somewhere around 1 mutation for every 10^7 to 10^8 replications. The use of a mutagen (mutation inducing agent) such as BU may increase the probability of mutation by a factor of 100 or 1000, but even so we are dealing with a very small fraction of mutational events (1 in 10^5

replications). In studying the effects of mutagens, therefore, it is necessary to use large populations. Microorganisms are especially suited for this because very large populations can be grown easily and very rapidly.

It is convenient, in determining whether a compound is a mutagen, to choose a test organism which is already a mutant and therefore is deficient in some enzymic activity. One determines whether the presumed mutagen can revert the original mutation, thereby restoring the missing enzymic activity.

Error of Incorporation: (BU incorporated as cytosine.)

Cytosine ⟶ Thymine (rather than guanine)
Error of Direction: (old strand BU occasionally acting as cytosine during replication)

Adenine ⟶ Guanine (rather than thymine)

Many species of bacteria can double every 20 minutes in a rich medium.

Exercises for Home, Desk and Lab (HDL's):

(1) Three types of mistakes possible in DNA replication are:

(2) A mutant DNA molecule can transfer a change in sequence down to the proteins. Trace this transfer.

(3) What evidence do we have in this chapter that the enzyme DNA polymerase is not specific?

(4) What are sections of DNA which specify a particular protein called?

(5) If a mutant bacterial strain lacks the ability to synthesize the amino acid alanine, under what conditions might this strain be healthily grown?

(1) a. Random insertion of wrong monomers
b. Addition of base pairs
c. Deletion of base pairs

(2) The mutant DNA would transfer the error to mRNA, thus affecting the sequence of a protein molecule.

(3) Bromouracil is able to fool DNA polymerase in cells grown in the presence of bromouracil so that it is incorporated into their DNA.

(4) Genes

(5) The mutant bacterial strain which lacks the amino acid alanine could be grown if that amino acid was supplied by an outside source, such as being supplied in the bacterial nutrient agar.

(6) 2AP is a mutagen. Base sequences would show a mutation if C rather than T is incorporated into proteins formed.

(6) The compound 2-aminopurine or 2AP can be mistakenly incorporated into DNA in place of adenine (A). During replication 2AP usually pairs with thymine (T), but sometimes with cytosine (C). Is 2AP a mutagen? If so, how could it induce a change in base sequence?

(7) No. It would be a phenotypic change only. It would not be inherited.

(7) Suppose that a medicine was developed for sickle-cell anemia which acted by directly changing the valine at position 6 in the mutant hemoglobin back to glutamic acid, so that the mutant hemoglobin is converted to normal hemoglobin. Would this induced change in the hemoglobin be a mutation?

ENERGY CAPTURE AND GROWTH

Outline: Energy Capture and Growth

SECTION	TOPIC	PAGE
Chapter I	Energy Capture	140
A.	Introduction	140
A.1	Experiment: Light and Plant Growth	141
A.2	The Sources of Carbon and Hydrogen Used in Photosynthesis	143
A.2.a	Experiment: Carbon Dioxide Uptake During Photosynthesis	143
B.	Quantity of Light in Photosynthesis	145
B.1	Experiment: Light Intensity and Oxygen Evolution	145
B.2	Optional Experiment: Relationship of CO_2 Concentration to Rate of Photosynthesis	147
C.	Light Quality and Photosynthesis	147
C.1	Experiment: Light Color and Photosynthesis	148
C.2	Experiment: Absorption Spectrum of Green Plant Pigments	149
C.3	Optional Experiment: Chromatography of Green Plant Pigments	151
D.	The Cell Powerhouse	153
D.1	Observation: Chloropiasts in Plant Cells	154
E.	Path of Carbon in Photosynthesis	155
E.1	Experiment: Photosynthetic Incorporation of $^{14}CO_2$ into Amino Acids	155

SECTION	TOPIC	PAGE
Chapter II	Energy Consumption and Metabolism	160
A.	Glucose Metabolism	161
A.1	Experiment: Growth of Yeast on Glucose	161
A.2	Experiment: Glucose Metabolism by Yeast Juice	167
A.3	Experiment: Glucose Metabolism by Boiled Yeast Juice	170
Chapter III	Metabolism and Genes	180

TEXT SECTION	ROUGH TIME ESTIMATES	EXPERIMENTS	DEMONSTRATIONS	TEACHING AIDS	OTHER STUDENT ACTIVITIES	PROBLEMS	OUTSIDE READING
Chap. 1. Energy capture		A.1 Light and plant growth					
	5 days	A.2.a CO_2 uptake during photosynthesis		Save dried Elodea for Exp. E.1.		1, 2, 3	
B. Quantity of light in photosynthesis		B.1 Light intensity and O_2 evolution		See page 154, T.G. for D.1.	Re-do Ex. B.1, standardizing the effects of heat on the manometer gas Ex. B.2	4	
C. Light quality and photosynthesis					Exercise 26 in Biology Expt. for High School Students, Amer. Cancer Soc., 1964 - "The Winogradsky Column"	5	
	1 day	C.1 Light color and photosynthesis				8	

TEXT SECTION	ROUGH TIME ESTI-MATES	EXPERIMENTS	DEMONSTRATIONS	TEACHING AIDS	OTHER STUDENT ACTIVITIES	PROBLEMS	OUTSIDE READING
		C.2 Absorption spectrum of green plant pigments			C.3 Chromatography of green plant pigments	6, 7	
D. The cell powerhouse	3 Days				D.1 Chloroplasts in plant cells. Exp. 3-5 Experimental Plant Physiology Joseph Arditti and Arnold Dunn; Holt-Rinehart-Winston		
E. Path of carbon in photosynthesis		E.1 Photosynthetic Incorporation of CO_2 in Amino Acids					
Chapter II Energy Consumption and Metabolism						1. 2	
	2 Days	A.1 Growth of Yeast on Glucose				3, 4, 5	
	2 Days	A.2 Glucose metabolism by yeast juice				6, 7, 9	
		A.3 Glucose metabolism by boiled yeast juic				8	

139b

Chapter 1: ENERGY CAPTURE

A. INTRODUCTION

Life requires energy. There is no alternative; energy is required for all the things a living system must do. Most animals, including humans, get this energy and the raw materials for growth by eating other animals and plants. Since the process of converting food into living material is less than perfectly efficient (recall the second law of thermodynamics), each step down the food chain sustains a smaller amount of life. Without an outside source of energy it is clear that the living community would soon starve itself. Ultimately there must be an energy input from a non-living source. That source is, of course, the sun, and its energy is converted into living material by photosynthesis. On land and sea this process takes place predominately in green plants. Therefore, they are the ultimate food producers for the entire living community.

For example it takes many pounds of feed to make one pound of cow, while the more efficient consumer, the bacteria E. coli, will make almost half a gram dry weight of E. coli from one gram of glucose.

The sun is also the ultimate source of coal, oil and hydroelectric power.

At least 60% of all photosynthesis takes place in the top layers of the ocean.

Only about 0.1% of the total incident light energy from the sun is captured via photosynthesis.

Most photosynthesis takes place in the oceans by single-celled organisms called algae or phytoplankton. The chemical reactions which occur during marine photosynthesis are almost

exactly the same as occur in land plants.

In both cases the plants use light as the energy source to build up the complicated polymers of which they are made. This very complex process we can investigate partially. For a start we can ask if light is necessary for plant growth.

A.1 Experiment: LIGHT AND PLANT GROWTH

Weigh two groups of bean seeds, each group containing 20 seeds. Plant the seeds in planters (20 in each) or in one-half gallon milk cartons. Since each carton should contain 5 seeds four cartons will be equal to one planter (group of twenty). One group of 20 seeds will be grown in the dark and the other group of 20 will be grown in a lighted area. When the second cluster of leaves is apparent on those plants growing in the light, remove both groups of plants (those growing in the dark and those growing in the light) and compare the relative growth rates by comparing the lengths. Then remove the soil, rinse away all of the soil debris and dry the plants (by groups) in an oven at approximately 100 degrees Celsius. After drying, weigh each set of bean plants and make an analysis based on the following:

Materials and Equipment

Bean seeds (40 per squad of students) Plant seeds at least two weeks prior to anticipated time of analysis

Planters or a number of ½ gallon milk cartons. (Cut the cartons down to a height of approximately six inches.)

The two groups of leaves should give a significant amount of photosynthesis so that a difference in the weight of the groups becomes apparent.

The student should obtain results that clearly demonstrate a weight difference between plants growing in light that actively photosynthesize and those plants growing in the dark that do not photosynthesize. These results should indicate the necessity of light for the photosynthetic process.

If there is no drying oven available, the plants may be dried using an infra-red or heat lamp.

If students question the non-drying of the original bean seeds, let some member of the class dry the bean seeds. The weight loss is about 10%.

1. Compare the dry mass of the plants growing in the light with those that have been in the dark.

1. Students should compare the <u>average</u> masses of the light and the dark groups. They may also wish to construct histograms.

2. Does the relative growth rate of bean plants seem to be affected by dark or light environment?

3. Using the information gathered in this experiment, define growth.

3. Growth = increase in dry mass.

4. If the bean seeds kept in the dark do not grow, how does one account for this relative period of no growth?

4. If the students feel that air "quality" is an important factor in the difference, then have them devise a set-up that circulates the same air by both sets of seedlings.

If it is found that light is necessary for growth, we can ask two questions:

1. What environmental materials does the plant incorporate in order to grow?
2. What colors of light promote plant growth?

Light will have many effects on plant development: the main point for the student to get is that <u>growth</u>, as measured by the increase in dry mass of the seedlings over the dry mass of the seeds, occurs only in the light.

The main elements are C, N, O, P and H, with a little S. C constitutes about 50% of the dry mass, N about 14%, O 25%, and H about 6%. P, S and salts

Answering the first question involves making some guesses and then testing them. From your

comprise the remaining 5%. On a mole percent basis, H is the most numerous element with C second.

Only as CO_2 or HCO_3^- and H_2O in the environment.

We use ^{14}C to measure carbon uptake by the plant, since we have only to measure how radioactive the plant becomes.

Properties of ^{14}C

The teacher may be worried about the hazards presented to the students by this isotope. Even if a student were to swallow the entire class sample of this isotope (10 microcuries) the radiation dose he would receive would be equivolent to a chest X ray. ^{14}C has a half-life of about 5000 years It's radiation is a relatively weak β^- (electron) particle with an energy of 0.16 MEV; i.e., this particle will penetrate a sheet of paper but will not go through a glass test tube or aluminum foil.

Materials and Equipment

10 microcuries of $NaH^{14}CO_3$
This is a license-exempt quantity of ^{14}C. It can be obtained from American Scientific Chemical, Portland, Oregon at a cost of $16.00.
Elodea (order enough Elodea for this and the next experiment) Carolina Biological Supply
Aluminum foil

reading in Chemistry of Living Matter what kinds of elements must the plant take up in order to make proteins, carbohydrates and nucleic acids? Let's concentrate on C and H for now. In what chemical forms were these elements available in the environment during your experiment "Light and Plant Growth?"

A.2 THE SOURCES OF CARBON AND HYDROGEN USED IN PHOTOSYNTHESIS

Our guess is that plants obtain their carbon from CO_2 and their hydrogen from H_2O. Let's test these hypotheses by measuring the uptake of radioactive carbon dioxide [$^{14}CO_2$] and the evolution of a gas (O_2) during photosynthesis.

A.2.a Experiment: CARBON DIOXIDE UPTAKE DURING PHOTOSYNTHESIS

Place a 15-centimeter sprig of Elodea in each of two (25 x 150 mm) test tubes. Completely cover one with aluminum foil so that light cannot reach the Elodea. Add enough 0.3% bicarbonate solution to each test tube to completely cover the plant. Take the test tubes up to the instructor,who will add several drops of radioactive bicarbonate solution to each test tube. Allow both test tubes to remain in a brightly lighted place for at least 40 minutes.

After a period of 40 minutes, wearing plastic gloves, pour the radioactive bicarbonate

solution from the test tube into a sink with running water. The Elodea should be rinsed thoroughly, placed on a paper towel and gently blotted. Wash the contents of the test tube down the sink and then rinse the test tubes carefully and thoroughly.

Place the Elodea back in the test tubes (be sure to label correctly) and dry overnight in a 100°C oven.

Prepare planchets carrying the dried sprigs according to your teacher's instructions. Determine the radioactivity of each sprig by ascertaining the counts per minute of ^{14}C. Save the dried sprig which was illuminated for experiment E.1.

1. Are both sprigs of Elodea significantly radioactive?
2. Is carbon incorporated into either Elodea sprig?
3. Is light necessary for the uptake of carbon?

An additional experiment involves collecting considerable gas from a large amount of Elodea photosynthesizing for perhaps 24 hours and then using the glowing splint test to show the gas is O_2.

With a little luck you have just shown that with light the plant converts $^{14}CO_2$ into some-

Plastic gloves
Geiger-Mueller counter or radiation level scaler
0.3% $NaHCO_3$ solution
Test tubes 25 x 150 mm.

Fresh, green, young sprigs of Elodea must be dark adapted (kept in the dark for 24 hours) or they will not perform at maximum efficiency. The "food" stored in the Elodea plant must be used up in the dark so that maximum photosynthesis can occur when exposed to light.

The teacher should have the student bring his or her test tube containing Elodea covered with 0.3% bicarbonate solution up to a central place where he will then add 1.0 microcurie of ^{14}C bicarbonate solution to each tube. To obtain a convenient working volume the teacher must dilute the radioactive bicarbonate solution with non-radioactive 0.3% $NaHCO_3$ solution to a concentration of about 5 μc/ml.

Before counting the $^{14}CO_2$ uptake in the leaves the student should first determine the background count per minute for a period of three minutes. One background count for the entire class is sufficient. The background count should be recorded on a data table in an appropriate column labelled CPM (counts per minute). Broiler-grade aluminum foil is suitable for making planchets. The latter are simply discs of foil of a diameter that will fit your counting set-up. These can be cut out with a razor blade or scissors. The dried Elodea sprig is very fragile and light.

Pulverize the dried Elodea against the bottom of the test tubes with a long stirring rod. Push the aluminum foil planchet over the mouth of the test tube forming a cap. Remove the cap, smear the inner side of the cap with Elmer's glue, place the cap back on the test tube and invert the test tube so that all of the Elodea pieces will fall into the cap. The cap (planchet) is now ready to be counted.

Results:

The tube covered with aluminum should show only a small increase in CPM's, if any, whereas the tube not covered should indicate an increase in CPM's of about 200. These results are presumptive for the uptake of carbon dioxide in the photosynthetic process. As bicarbonate is the only tagged material the interpretations are obvious.

thing in the plant and that a gas is given off in the process (try splint test). The C_2 prcbably comes from the splicting of H_2O.

$$2H_2O \xrightarrow[h\nu]{} 2\ [\ H\ [\ + 2H^+ + O_2$$

But did your experiment prove that H_2O was the source of oxygen? What the $^{14}CO_2$ is converted into is still another mystery which we will try to solve in another experiment.

First, however, let's look more closely at the light involved. For a start we will try to find out about light and photosynthesis, varying first light intensity and then wavelength.

B. QUANTITY OF LIGHT IN PHOTOSYNTHESIS

B.1 Experiment: LIGHT INTENSITY AND OXYGEN EVOLUTION

Set up the equipment as shown in Figure B.1. Place Elodea and sodium bicarbonate solution in the test tube with the solution exactly half filling the test tube. With the clamp removed, carefully insert the stopper as tightly as possible. Attach the long rubber tubing to the manometer and place the test tube in the Erlenmeyer flask with water.

Place the lamp at a distance of 40 cm from Elodea. Be certain to measure the distance between the test tube and the light bulb and not the base of the lamp. Allow the apparatus to stand for five minutes before placing the clamp

Use the apparatus illustrated in Fig. B.1, modified by replacing the manometer by a test tube filled with water.

The exact chemical nature of the hydrogen / H / produced is complicated, but essentially it is in the form of a strong reducing agent.

No. It could have come from the CO_2 or somewhere else. Experiments have shown that the source was H_2O, by using water labeled with the heavy isotope ^{18}O and showing that the O_2 evolved was $^{18}O_2$.

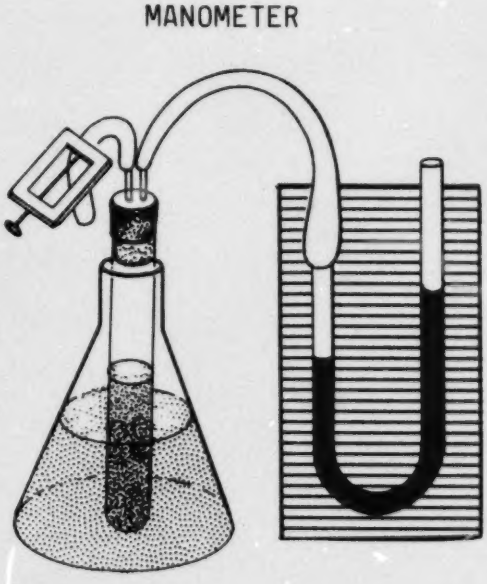


Figure B.1

Materials and Equipment (for each squad)

1 sprig of Elodea about 15 cm long
1 Erlenmeyer flask (250 ml)

on the short rubber tube. This is done in order to allow the solution to become saturated with oxygen. Fasten the clamp. Tap the test tube before each reading in order to free any trapped bubbles of oxygen.

Immediately observe and record the reading of the manometer. The reading may be made at either end of the manometer tube, but all readings must be made thereafter at the same end of the manometer tube.

Take manometer readings at two-minute intervals until the rate has been stabilized, that is, until the same amount of oxygen is evolved during at least three consecutive two-minute intervals.

Once the rate of oxygen evolution has been stabilized, record the rate. Move the light source to a distance of 20 cm from the test tube and repeat steps 3 and 4 of the above procedure.

When the rate has been stabilized at a distance of 20 cm, record the rate. Move the light source to a distance of 10 cm from the test tube. Repeat the above procedure and record the rate at 10 cm.

Plot the data, using the distances traveled (mm) by the colored solution in the manometer as the vertical axis and time in minutes as the horizontal axis.

1% sodium bicarbonate 100 ml
Meter stick
Lamp with 100 watt bulb
Masking tape
Test tube, 25 mm x 150 mm
1 two-holed stopper to fit test tube
2 pieces of glass tubing each about 6 cm long
1 piece of rubber tubing 5 cm long
1 clamp
1 piece rubber tubing 10-15 cm long
1 manometer tube (glass) bent to form a "U" with each arm 20-24 cm long (inside diameter of manometer tube 2-3 mm)
Colored water or food coloring to put in manometer to height of 3-4 cm in each arm (add several drops of detergent solution)
1 piece of cardboard, approximately 25 x 10 cm, which is stapled to a sheet of millimeter-ruled graph paper as background for the manometer tube.

NOTE: Oxygen production is proportional to light intensity only at low light intensity. If the light intensity gets too high, then oxygen production is limited by other factors like CO_2 availability, etc.

1. *mm of O_2/unit time*

2. *To allow for equilibration of O_2 and temperature (water has to become O_2-saturated before O_2 gas production is constant).*

B.2 *Optional Experiment: RELATIONSHIP OF CO_2 CONCENTRATION TO RATE OF PHOTOSYNTHESIS*

Set up the apparatus as in Experiment B.1 with the light 10 cm from the Elodea. Submerge the Elodea consecutively in the following concentrations of sodium bicarbonate solution and measure the rate of oxygen evolution in each: 0%, 0.25%, 0.5%, 0.75%, and 1.0%.

One sprig of Elodea usually will "wear out" before all concentrations are used. There are several ways to circumvent this problem, such as using several sprigs of Elodea. You could discuss this with the class and elicit the various methods of standardization which could be applied to normalize results from different sprigs.

Construct a graph showing the relationship of rate of oxygen production (vertical axis) and carbon dioxide concentration (horizontal axis).

Remember:

$E = h\nu$ so which color of light has the most energy? The least energy?

1. How did you measure the rate of photosynthesis?

2. Why is it necessary to wait five minutes before taking readings on the manometer?

C. LIGHT QUALITY AND PHOTOSYNTHESIS

The effect of light quality on photosynthesis can be investigated if we separate light into its component colors. Then we could illuminate different plants with different colors of light and determine which grow and which don't. A prism separates white light into its colors. The colors and their wave lengths are:

Color	Wavelength Range λ	Frequency Range ν
violet	3800-4200 Å	8×10^{14} waves/sec.
blue	4200-4900 Å	
green	4900-5350 Å	
yellow	5350-5900 Å	
orange	5900-6400 Å	
red	6400-7000 Å	4×10^{14} waves/sec.

Light of wavelengths shorter than 3800 Å or longer than 7000 Å is invisible to the human eye; the first is called ultraviolet radiation and the second infrared radiation. Plants use light in the visible spectrum for photosynthesis. A practical way of separating out the color wanted is

to use colored cellophane. For example red cellophane looks red because it allows only light in the wavelength region 6400-7000 Å to pass through. Thus we could wrap our plants in various colors of cellophane and expect to get only the color of light transmitted by the cellophane. Then we could measure either total growth as before, or we could follow CO_2 uptake or O_2 evolution as in the second experiment; these latter measurements would be faster than total growth.

C.1 Experiment: LIGHT COLOR AND PHOTOSYNTHESIS

Set up the apparatus as in Experiment B.1. Wrap colored cellophane around the Elodea test tubes. Place the Elodea at a distance from the light given by your teacher.

Measure the rate of oxygen evolution. Construct a graph showing the relationship of oxygen production (vertical axis) and light color (horizontal axis).

The experiment shows us that some wavelength ranges are much more effective than others in causing photosynthesis and in particular that green light is most ineffective. Perhaps this is why most photosynthesizing plants are green. They don't use the green light, and therefore it is not absorbed. Let's now turn the argument around. Light that is effective in photosynthe-

The teacher could set up a Winogradsky column for the growth of other kinds of photosynthetic organisms--bacteria and algae; this is a low-work experiment which the student should enjoy. See exercise 26 "The Winogradsky Column - a Microbiological Evolutionary Totem Pole" in Biology Experiments for High School Students, American Cancer Society, 1964.

Materials and Equipment (for each squad)

All the materials of Experiment B.1, plus
Pieces of cellophane (red, yellow, green, blue)
1 light meter for the whole class

Since each test tube of Elodea must receive the same light intensity (explain to students) the distance of the beaker from the light source must vary. Place the red cellophane over the light meter. Hold the red cellophane-covered light meter 10 cm from the light source and read the meter. Remove the red cellophane. Place two layers of green cellophane over the light meter. Move the green cellophane-covered meter from the light source until the light meter reads the same as it did with the red cellophane. Record the distance. Repeat with the blue and yellow cellophanes. The cellophanes are now

calibrated relative to each other, as to the amounts of light energy transmitted.

When the students run the experiment, they should place their cellophane-covered test tubes at the calibrated distances.

Because of the time involved each team of students should use cellophane of only one color. Place their data on the board. Have each student plot all of the class data. It is therefore important that each team use an Elodea sprig of the same size.

Materials and Equipment (for one student)

Elodea (15 cm sprig)
Acetone (20 ml)
Mortar and pestle
Test tubes (13 x 100 mm)

The critical step in this exercise is the extraction of the chloroplasts. The mortar and pestle provides the easiest method. However a suitable extract can be obtained by macerating the Elodea leaves in acetone in a test tube, using a stirring rod. Decant the liquid into another test tube and take readings in the spectrophotometer. A blender, if available, can also be used to prepare the extract.

The student should plot the spectrum and get a curve showing a primary absorption peak in the blue-green, green region and a secondary

sis must be absorbed by something in the plant. Absorption means the capture and conversion of the energy that is the light. We might guess that the capturing agent is some kind of molecule or molecules. If so we should be able to separate it out of the plant material using chemical techniques.

C.2 Experiment: ABSORPTION SPECTRUM OF GREEN PLANT PIGMENTS

Place a 15 cm sprig of Elodea in a mortar, add to it 10 ml of acetone and grind the Elodea using a pestle. Save the supernatant solution for this experiment and the next one. Take readings as indicated below at successive 20 millimicron intervals on the spectrophotometer. (See instructions below.)

A spectrophotometer can be used to make precise measurements of pigment absorption of specific light wavelengths over the entire visible spectrum. From these data a plot of the absorption spectrum can be prepared. Because of the variation in the composition of pigments from plant to plant, as well as differences in different measuring instruments, the plot you are to prepare will perhaps differ from the one you will see in general textbooks.

The student should get detailed instructions on the use of the spectrophotometer from the

instructor. Briefly, the general outline is as follows:

(1) Turn on the instrument and allow five minutes for warm-up.

(2) Set the zero point on the transmittance scale with nothing in the sample holder.

(3) Fill one of the special spectrophotometer tubes with the acetone solvent being used (to be called the blank) and insert this in the sample holder. Turn the wavelength control to 430 millimicrons, and set the absorbency at zero with the light control. (You will have to repeat this blank setting for each new wavelength measurement, since the solvent itself has its own absorption spectrum for which compensation must be made, and because the different wavelengths emitted by the lamp have different intensities.)

(4) Prepare a very dilute sample of your extract by adding a few drops to a spectrophotometer tube half-filled with the solvent. Adjust this sample by addition of solvent or extract so that it has an absorbency of about 0.7 at 430 millimicrons.

peak in the red, indicating zones of greatest absorption. The flat segments of the curves indicate regions of high transmittance.

These instructions are for a Bausch & Lomb Spectronic 20 Spectrophotometer. More complete instructions for use of the instrument are given on pp. 202 ff of Arditti and Dunn's Expt'l Plant Physiology.

(5) Now set the wavelength at 380 millimicrons. Using the blank solvent tube, set the scale at zero absorbency. Insert the pigment extract sample and read the absorbency on the meter. Repeat these steps, taking successive readings at 20 millimicron intervals up to 700 millimicrons. Before changing to each new wavelength setting, turn the light control down (counterclockwise) to avoid overloading the photocell.

The data should be reported on a report sheet. Graph the results with light absorption on the vertical axis and wavelength on the horizontal axis.

On the same piece of graph paper, plot the results of Experiment C.1: LIGHT COLOR AND PHOTOSYNTHESIS. Explain the relationship between the two sets of data.

C.3 Optional Experiment:
CHROMATOGRAPHY OF GREEN PLANT PIGMENTS

Materials and Equipment
(for one student squad)

Acetone extract from C.2
1 beaker (250 ml)
1 chromatostrip
Benzene 7 ml
Acetone 3 ml
1 TLC plate Baker-flex silica gel 1B. Use a piece 10 cm x 2½ cm,

The acetone extract left over from Experiment C.2 should be concentrated by evaporating the acetone by means of a hot water bath until only about 1 ml is left.

Place 1 drop of the leaf pigment extract 1.5 cm from one end of a chromatostrip. Place

the strip vertically with the spot end down in a 250 ml beaker containing a 1 cm layer of benzene: acetone, 7:3 v/v. Cover with a watch glass or aluminum foil.

which is ¼ of a full sheet.
J.T. Baker Chemical
Boker-Flex Silica Gel 1B
Flexible TLC Sheet
(5 x 20 cm Box of 50
$13.50)
Also used in Exp. E.1

The solvent rapidly rises through the thin layer of adsorbent. Separation of the pigments is apparent almost immediately. Allow the solvent to rise until it reaches 1 cm from the top of the strip. Remove the strip from the solvent and observe the pigments.

Lower percentages of acetone in the development solvent can be used for better separation of chlorophyll a and b. If the pigment spots are too dim, extract a greater quantity of pigment with the same amount of solvent as the original extract.

You should note that a yellow band (carotenes) moves about as fast as the solvent front. Lower on the paper will be one or more yellow (xanthophyll) bands, a bluish-green (chlorophyll a) band and a yellowish-green (chlorophyll b) band, in that order. Identify and outline these pigment bands with pencil and save your chromatograms for later discussion.

The accessory pigments (carotenes and possibly xanthophylls) also function in the photosynthetic process. They may help trap light energy at wavelengths at which the chlorophylls don't absorb. They may also function in the transfer of the energy to the site(s) of the photochemical reactions (see following section).

The green bands on your chromatograms are the chlorophylls a and b. Both your action spectrum for photosynthesis and your absorption spectrum for the leaf pigments will have large bumps

or peaks in the wavelength regions 4000-4800 Å (blue) and 6200-6800 Å (red). If you had chromatographed enough pigments you could have recovered the pure chlorophylls from the chromatogram and done light absorption spectra on them. These spectra would have shown peaks in the same two wavelength regions. Still, even if there is a suspicious resemblance between action spectrum of the plant and the absorption spectrum of the chlorophyll, we can't really say that the pigment is necessary for photosynthesis. In fact, many other experiments do show that chlorophylls are the primary photosynthetic pigments; that is, they are the agents which capture light energy for photosynthesis.

D. THE CELL POWERHOUSE

All of the photosynthetic pigments are confined in tiny particles within the plant cell. These particles are called chloroplasts. They are the powerhouse for the cell, capturing light energy and using it in two reactions.

(1) $2H_2O \xrightarrow[\text{chloroplasts}]{h\ \nu} 2\ [H] + 2H^+ + O_2$

where 2 [H] is "reducing power."

(2) $ADP + H_3PO_4 \xrightarrow[\text{chloroplasts}]{h\ \nu} ATP + H_2O$

where ATP, as you already know, is "activating power."

More correctly written reaction (1) is

$$2NADP + 2H_2O \xrightarrow[chloroplasts]{h\ \nu} 2NADPH + 2H^+ + O_2.$$

The NADPH (reduced nicotinamide adenine dinucleotide phosphate) is a reducing agent common to all living things.

These reactions are often called the light reactions of photosynthesis. The reducing and activating chemicals produced in the light are then used in "dark" or light-independent reactions to chemically convert CO_2 into cellular materials.

Chloroplasts can be easily seen in plant cells under the microscope, especially if the plants are single-celled algae.

D.1 Observation: CHLOROPLASTS IN PLANT CELLS

Place a small drop of algae culture on a microscope slide and lay a coverslip onto the drop of liquid: observe through a microscope first at low power (100 - 150 x), then at higher power (250 - 400 x). Is the green color spread throughout the cells or localized? Try observing chloroplasts in leaves. Can you slice the leaves with a razor blade so as to obtain a single layer of cells?

It is quite easy to isolate chloroplasts from cells. If the isolation is done gently and quickly, the particles will carry out ATP generation and evolve O_2 when exposed to the light. Purified chlorophyll will not do these things. It appears that in addition to chlorophyll, some part of the chloroplast structure is necessary for the light reactions.

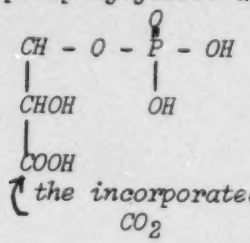
Obtain 2-5 days in advance a culture of Euglena or Chlamydomonas from Carolina Biological Supply, or obtain some water from a pond having a green scum on it. Keep any or all of these on a window sill out of direct sunlight until used.

After a few trials, it is usually possible to obtain a single cell layer by cutting through the leaf at an angle.

Two supplementary experiments which could be done here or later by advanced students are O_2 evolution by chloroplasts in the light and ATP generation by the same. These are written up in experiment 3-5 in Experimental Plant Physiology, by Joseph Arditti and Arnold Dunn (Holt-Rinehart-Winston). (Nearly half of this book is con-

prised of concise appendices on radioactivity, colorimetry, pH and a variety of other topics which could prove generally valuable to the teacher.)

3-phosphoglyceric acid



Materials and Equipment (for one student)

1 test tube (13 x 100 mm or larger)
1 beaker (25 ml)
Planchets (small aluminum foil, 2"x2")
1 watch glass or aluminum foil
1 TLC plate Bakerflex silica gel 1B cut in half so sheet is 10 x 5 cm
1 micropipet (draw out melting point capillary)
80% (v/v) ethanol (1 ml)
Development solvent (1) chloroform: methanol: 7.5M NH_4OH (2:2:1 v/v). Another solvent system which works well is (2) n-butanol: conc. NH_4OH (67:33 v/v)
Difco casamino acids solution 0.1% (w/v)
Ninhydrin spray: 0.2 g ninhydrin in 100 ml n-butanol (use in Windex spray bottle); use as a dip if no sprayer
1 pair plastic gloves
1 G.-M. tube with scaler

E. PATH OF CARBON IN PHOTOSYNTHESIS

The first product made from CO_2 during photosynthesis is 3-phosphoglyceric acid. As will be explained later (see diagram of EMP Pathway, page 172) this compound can be metabolized in several ways, so as to ultimately make amino acids (for proteins) and glucose (for starch) as well as the monomers for nucleic acids. For the moment let's simply determine if radioactivity from $^{14}CO_2$ gets into amino acids.

E.1 Experiment: PHOTOSYNTHETIC INCORPORATION OF $^{14}CO_2$ INTO AMINO ACIDS

Place the dried Elodea from Experiment A.2.a in a test tube. Add enough 80% ethanol to the test tube to cover the Elodea and place the tube in a boiling water bath for two minutes.

Obtain a piece of thin layer chromatography (TLC) material from your teacher. Draw a light pencil line about 2 cm from the end of the TLC plate. Wearing plastic gloves and using a micropipet, draw up some of the Elodea extract and make a thin streak of pigment along the pencil line. Allow this to dry. Repeat this step several times until a dark green color appears on the plate. It is important to get a strong concentration of pigments in one neat streak. On another TLC plate, streak the casamino acids solution in the same way as you did the Elodea

extract. This will be a comparison plate which will show the locations of all the amino acids.

Place enough development solvent in a 250 ml beaker to just cover the bottom of the beaker but not to cover the pigment streak on the TLC plate. Place the TLC plates in the beaker with the streak of green or amino acids at the bottom and the TLC plates leaning against the side of the beaker. Cover the beaker with aluminum foil or a watch glass. Allow the development solvent to rise up the TLC plates to within 1 cm of the top of the plate. Remove the TLC plates. Allow to dry until there is no odor of ammonia. Spray or very quickly dip the plates in ninhydrin solution (wear gloves!). Dry the plates in an oven at 90° until purple streaks appear. Each purple streak that appears is due to an amino acid. Cut out the streak(s) from the Elodea extract plate, place each streak on a separate planchet and count with a radioactivity counting device.

Warning: Ninhydrin reacts with skin protein to give a permanent blue color. Color will wear off in two to six weeks.

Exercises for Home, Desk and Lab (HDL's)

(1) The sun, which is the ultimate source of energy, has its energy converted into living material by photosynthesis. Where does most photosynthesis occur?

(1) In phytoplankton in the oceans.

(2) a. Nitrogen, hydrogen, carbon and oxygen.

b. Carbon, hydrogen and oxygen.

(3) CO_2 is the source of carbon, and H_2O is the source of hydrogen.

(4) a. The temperature may increase slightly.

b. As the temperature increased the expansion of the trapped gas would likely be greatly affected.
c. No.

d. Determine the volume change in the manometer using distilled water in the test tube rather than the $NaHCO_3$ solution.

(5) a. Red and yellow
b. Green

(6) a. Yellow--carotene

(2) a. Proteins are made up of what kinds of elements?

b. Carbohydrates are composed of what elements?

(3) What is the source of carbon and hydrogen in plants?

(4) In Experiment B.1: Light Intensity and Oxygen Evolution

a. What effect might the lamp at a distance of 10 cm from the test tube have on the temperature of the sodium bicarbonate solution during the length of that one trial?

b. What then would the effect be on the gas trapped in the top of the test tube and manometer?

c. Is this compensated for during your experiment.

d. What would have to be done in order to account for this amount of error?

(5) a. What colors of light are effective in photosynthesis?

b. What color of light is least effective in photosynthesis?

(6) Paper chromatography shows four color bands due to the solvent elution.

a. List the band color and the corresponding

pigment molecule, starting from the top of the chromatography paper.

Yellow--xanthophyll
Blue-green--chlorophyll a
Yellow-green--chlorophyll b

b. If you are provided with the given information that small molecules are carried at a faster rate than large molecules, which of the four molecules listed above is the largest?

b. Chlorophyll b

(7) Where are photosynthetic pigments located within the cell?

(7) Chloroplasts

(8) Can plants convert CO_2 into cellular materials during the night? Explain.

(8) Yes, the reducing and activating chemicals produced during the day are used in light-independent reactions to chemically convert CO_2 into cellular material.

Chapter II: ENERGY CONSUMPTION AND METABOLISM

Consumer organisms are those that feed directly on other organisms. Animals, of course, are all consumers. They convert either plant material or other animals into raw materials for their own use. Also they obtain their energy from the chemical energy stored in their food. This chapter is concerned with some of the details of these processes.

The first problem of the consumer is digestion of polymers down to monomers. This requires special enzymes. You will recall there are proteolytic enzymes that split proteins to amino acids, and there is an enzyme in saliva that converts starch to glucose. There are also enzymes which convert nucleic acids to nucleotides. There are several reasons why polymers must be broken down before they can be used.

First, remember that proteins and nucleic acids of every kind of organism are specific - different from those in any other living thing. Second, such large molecules as proteins and nucleic acids cannot pass through the cell membrane. If cell membranes permitted such molecules to get in easily they could also get out easily,

Actually every individual is unique. That is to say, at least one of his proteins will differ in sequence from the homologous protein of every other individual of his species. However, on the average a given protein from an individual will be identical to the same protein from another individual of his species. This apparent contradiction arises because of the

large number of proteins in each individual.

d-Glucose, often called dextrose, is often used by athletes for a quick supply of energy.

Since mammals, including human beings, can't perform all of the chemical steps required to convert glucose into everything needed, vitamins, amino acids and minerals are needed. Simpler consumers, such as bacteria, molds and yeast, can make everything from glucose.

For Growth of Yeast on Glucose:

Materials and Equipment (for each student squad)

Pinch of active dry yeast (Fleischmann or Red Star brands)
Test tubes
2 25 x 150 mm
4 18 x 150 mm
4 13 x 100 mm
4 10 x 75 mm
4 125 ml Erlenmeyer flasks
1 5 ml pipet

Solution A: (25 ml per squad) to make 1 liter
$MgSO_4$ 0.05 g $(NH_4)SO_4$ 1.0g
sodium citrate·$5H_2O$ 0.25 g
citric acid 0.25 g
tap water 1000 ml

and that would be a disaster for the cell (and the organism).

All chemical and physical processes continuously going on in living cells taken together constitute metabolism. Not only are polymers being broken down but new monomers and polymers are being synthesized. In addition, another very important process involving the release of usable energy from glucose in the form of ATP takes place. Glucose is one of the best foods for almost all living things. It is quickly and easily converted to usable energy and also yields necessary raw material for cell growth.

A. GLUCOSE METABOLISM

We will concentrate our laboratory work on glucose metabolism.

A.1 Experiment: GROWTH OF YEAST ON GLUCOSE

Add a very small pinch of dried yeast to a 25 x 150 ml test tube containing 21 ml of solution A. Allow the dried yeast to soak for a few minutes. Place one drop of the soaked yeast upon a microscope slide and examine with the high power objective of a microscope. If there are too many yeast organisms in the field of vision dilute the yeast culture with solution A until there are 20 to 40 yeast cells in the

high power field of view of a single drop of diluted culture.

Use this diluted yeast culture to prepare your experimental cultures as indicated in the protocol table Yeast Growth.

Leave these yeast cultures at room temperature overnight. Examine one drop of each culture under the microscope. You will have to use a long eyedropper to obtain a drop of culture from culture 4. Relative cell densities should be about

100 (3) : 10 (4) : 1 (1 & 2).

Note the differences in cell density between the cultures when started and one day later. Try to obtain estimates of the relative cell densities in each culture; for example culture 3 may be ten times as dense as culture 4. Is there any obvious indication of growth (cell multiplication) in cultures 1 or 2?

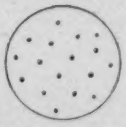
To each of the NaOH solution tubes taken from your cultures add 4 ml of $BaCl_2$ solution. What do you observe in each case?

Transfer 3 ml of each culture to a numbered centrifuge tube and centrifuge down the yeast cells. Carefully pour off the clear supernatants and add one drop of each to 3 ml of alcohol test solution. Also try a drop of the 10%

Boil for ten minutes or autoclave. Note: $MgCl_2$ and NH_4Cl at the same w/v concentrations can substitute for $MgSO_4$ and $(NH_4)_2SO_4$ if you also add a pinch of Na_2SO_4.

Heavy mineral oil, 50 ml per squad. Add approx. 1 tablespoon of liquid cooking oil per liter of mineral oil.
10% (w/v) glucose 1 ml per squad (autoclave)
1% (w/v) $BaCl_2$: 16 ml per squad
(Keep stock solution tightly closed).

The microscope field of vision should be something like:



If the yeast is so concentrated that cells touch, then students will not be able to see any increase in the number of cells.

For phosphate buffer solution pH = 6.0, mix:

(1) 214.0 ml of 0.0025 M KH_2PO_4 solution

(2) 86.0 ml of 0.0025 M K_2HPO_4 solution

(Should be autoclaved if you wish to store for a week or more.)
Note: Absolute sterility is not required for solution A, glucose or phosphate solutions, nor must students use sterile techniques during the preparation and handling of their yeast cultures. Yeast grows fast enough that there should be no problems from bacterial or mold contamination during

Protocol: Yeast Growth

Reagent \ Culture #	1	2	3	4
Diluted Yeast in solution A	5 ml	5 ml	5 ml	5 ml
10% Glucose solution	one drop	———	one drop	one drop
1M Phosphate solution	———	one drop	one drop	one drop
Container to be used for this culture	foil capped 125 ml Erlenmeyer See diagram (1) below.	as for culture #1	as for culture #1	see diagram (2) below

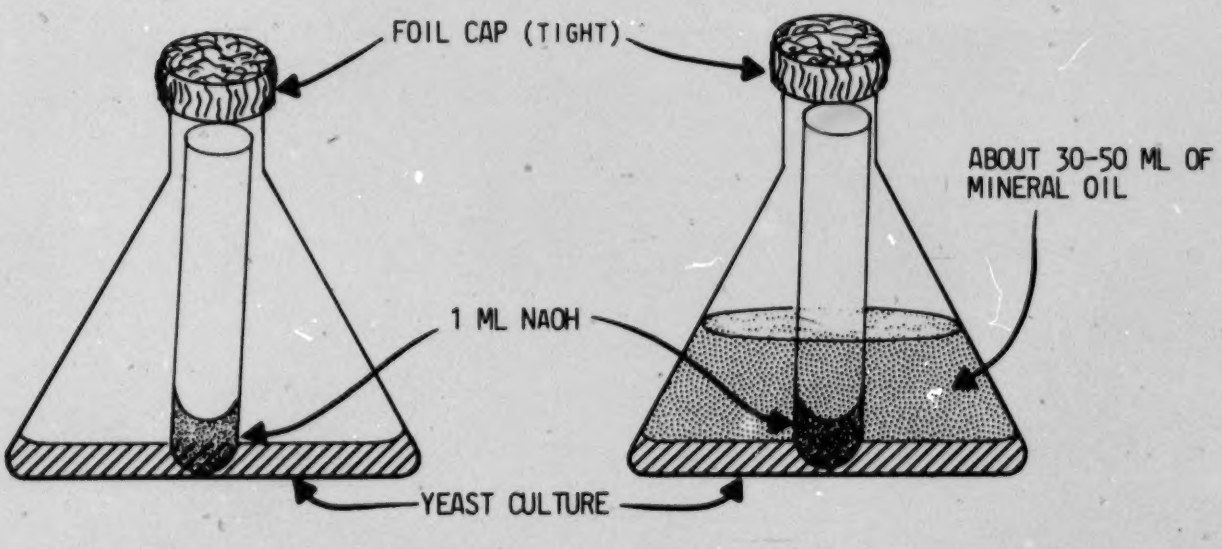
Table A.1

Diagram 1

After the yeast culture is in a flask, carefully put into the flask a 10 x 75 mm test tube containing 1 ml of 1 M NaOH solution as indicated below

Diagram 2

Prepare culture 4 exactly like 1-3, but before putting in the NaOH test tube carefully fill flask with mineral oil as indicated



CULTURES 1, 2 AND 3

CULTURE 4

standard ethyl alcohol solution provided by the teacher. A color change from yellow to red indicates the presence of ethanol. Which of your yeast cultures made ethanol?

You will have found that in the presence of O_2 (culture 3) glucose is almost completely consumed, the products being more yeast cells and CO_2. In the absence of oxygen (culture 4) the glucose is much less efficiently used. That is, fewer yeast cells are made and the glucose is partly converted to ethanol (CH_3CH_2OH or C_2H_5CH). The overall reactions are:

aerobic degradation (with O_2):

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ + usable energy

(glucose)

anaerobic degradation (without O_2):

$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$ + usable energy

(glucose) (ethanol)

Aerobic degradation looks very much like photosynthesis run in reverse. Photosynthetic energy stored in glucose is converted into usable energy (ATP) as the glucose is broken down into CO_2 and H_2O. You will remember that photosynthesis starts with these simple molecules and by adding light energy converts them into glucose, other monomers and O_2. This chemical and energy interdependence of produc-

the experiment. The small amount of unsaturated cooking oil is introduced into the mineral oil to provide the oleic and linoleic acids which are required by yeast during anaerobic growth.

The precipitate formed is $BaCO_3$; the amount obtained should be a rough measure of the amount of CO_2 produced in each culture.

Also H_2O

Alcohol Test Solution
40 g $(NH_4)_2$ [$Ce(NO_3)6$] dissolved in 100 ml of 2N HNO_3. Immediately before use dilute ten ml of this stock solution with 20 ml of water.*
**Ceric ammonium nitrate*

10% standard (v/v) ethanol: 10 ml of ethyl alcohol (denatured ok) plus H_2O to 100 ml total volume.

How much more efficiently was glucose used for growth when O_2 was present?

ers and consumers is vital to life as we know it. Thus, the chemical elements composing life are continually reused and recycled in the overall conversion of light energy into life.

These summary equations show only the input (glucose) and the final products. They don't show the many chemical steps in between. Also they account only for the glucose catabolized to provide energy, although obviously part of the glucose was converted into more yeast cells.

There are 11 enzymatic steps between glucose and ethanol. If O_2 is present the complete combustion of glucose requires 10 additional steps.

Those steps or pathways that yield energy are termed catabolic. Those that synthesize monomers are called anabolic. In some cases there is not a clear distinction between the two kinds of metabolic activities.

The degradation of glucose by yeast in the absence of oxygen is a very important process, which has the name fermentation. It is the process used to make wine and beer; also it is used in making bread. What product of glucose fermentation is important in making wine or beer? In "raising" bread dough?

It was a great surprise to scientists when they found that they could completely grind up yeast cells and that the "juice" obtained would also ferment glucose. This discovery was the beginning of classical biochemistry. Classical biochemistry is primarily concerned with discovering and understanding the intermediate chemical steps of metabolism and their interrelationships. We will now examine some of these intermediate chemical steps.

NOTE THE DIFFERENCE IN AMOUNT OF ATP GENERATED, AEROBIC VS ANAEROBIC RESPIRATION.

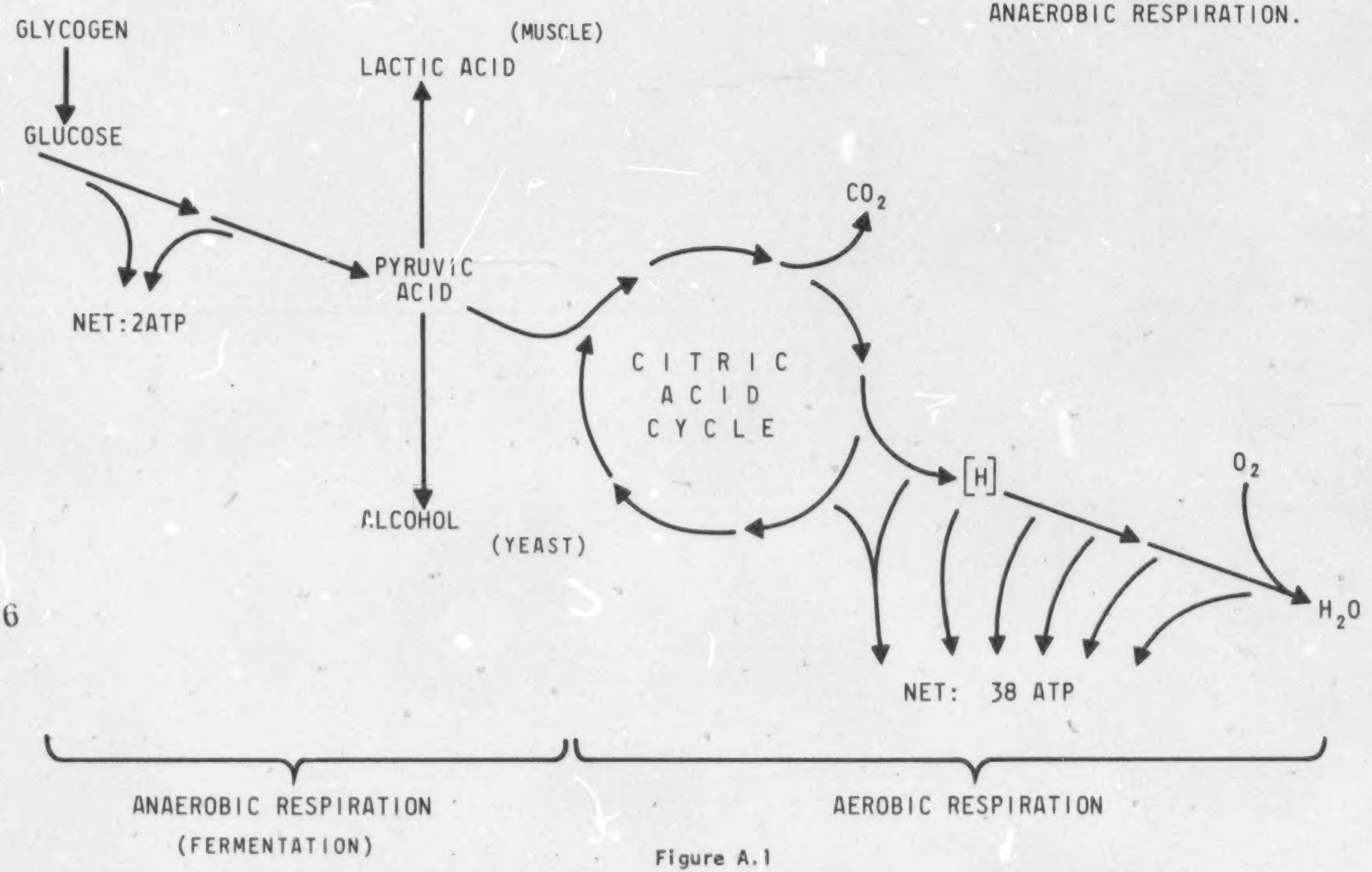


Figure A.1

Materials and Equipment
(for a class of 30 students)

0.1 M potassium bicarbonate 800 ml
Active dry yeast, 28 packets or 7 oz.
0.2 M fructose-1, 6-diphosphate, 150 ml
1.0 M d-glucose (dextrose), 200 ml
1.0 M potassium phosphate buffer pH 6.9. To prepare mix:
(1) 206.0 ml of 0.02 M KH_2PO_4 solution
(2) 94.0 ml of 0.02 M K_2HPO_4 solution
Manometers, see Ex B.1, ch.1
60 test tubes (any test tube size from 18 x 150 mm-25 x 200 mm will do)
Blender, one or more
Dish pans or pneumatic troughs, 15
Clinical centrifuge and 15 or more centrifuge tubes
125 ml Erlenmeyers, 15
Firefly Lantern Extract
Note: The amount of yeast noted in student guide provides enough juice for two-student squads. However, it is the minimum volume necessary to cover the blaces in most blenders and therefore the minimum volume in which efficient rupture of the yeast cells will occur.

The teacher may wish to prepare the yeast juice in advance if time or blenders are in short supply.

The juice can be saved overnight in a refrigerator either before or after the preincubation with glucose and fructose-1, 6-diphosphate.

Step (3) is needed in order to deplete the

A.2 Experiment: GLUCOSE METABOLISM BY YEAST JUICE

(1) Work in squads as indicated by your teacher. Blend 1 ounce (4 packets or about 28 g) of dry yeast in a blender with 80 ml of 0.1 M potassium bicarbonate for 1 minute. Allow the blended material to stand for five minutes and then blend again for 1/2 minute.

(2) Pour the blended liquid into centrifuge tubes, make sure that the centrifuge is balanced and centrifuge at a maximum speed for 15 minutes. Then carefully siphon off the cloudy liquid layer with a pipet. It is not necessary that you get all the liquid, and it is better if you don't pick up any of the solid material. Put all of the liquid - the yeast juice - into a 125 ml Erlenmeyer flask.

(3) To the yeast juice add 8 ml of 1 M glucose and 10 ml of 0.2 M fructose-1, 6-diphosphate. Incubate this mixture by placing the flask in a large beaker of warm (35-40°C) water. Keep it there for 30 minutes or until it begins to bubble vigorously (CO_2 gas). Then store the juice in a refrigerator if class time is up.

(4) Using this preincubated yeast juice, set up 4 manometer tubes like that shown in

Figure B.1, Chapter I (Energy Capture). Set up these manometers as indicated in Protocol: Yeast Juice. A very light film of grease (lanolin or vaseline) should be applied to the stoppers to ensure an airtight seal. If the apparatus leaks it is of no value.

(5) After assembling the entire apparatus, place the test tubes in a rack in a room temperature water bath (a plastic dishpan will do). Let them reach temperature equilibrium with the water bath (3 to 5 minutes) and then close the clamps and start manometer readings.

(6) Each manometer should be read at 5 minute intervals. Between readings, the test tubes should be shaken gently every 30 to 60 seconds to release all CO_2 from the liquid. Record your readings in a table something like this:

Manometer Readings

Time	#1	#2	#3	#4
1:35	10			
1:36		12		
1:37			9	
1:38				13
1:40	13			
1:41		14		
1:42			13	
1:43				21
		etc.		

inorganic phosphate present in the yeast juice, so that the requirement for same in glucose metabolism can be demonstrated.

Sources for special materials:

(1) Schwarz Bioresearch, Inc.
Orangeburg, N.Y. 10962
#4214-23, Fructose-1, 6-diphosphate
Monomagnesium salt at Tech grade $8/25 g

(2) Sigma Chemical Co.
3500 De Kalbst
St. Louis, Mo. 63118
FLE-250, buffered Firefly Lantern Extract; extract from 250 mg lanterns, $9.

The main points for the student to grasp are:

(1) That an intact living cell is not needed to catalyze glucose breakdown;
(2) Inorganic phosphate is needed in stoichiometric quantities.

Protocol: Yeast Juice

Reagent \ Manometer #	1	2	3	4
Preincubated Yeast Juice	5.0 ml	5.0 ml	5.0 ml	5.0 ml
1.0 M potassium phosphate pH 7.0	———	0.3 ml (6 drops)	0.05 ml (1 drop)	0.3 ml
1.0 M glucose	1.0 ml (20 drops)	———	1.0 ml	1.0 ml
Distilled H_2O	0.3 ml (6 drops)	1.0 ml	0.3 ml	———

Table A.2

Read each manometer for at least 30 minutes. In which is CO_2 production greatest; least?

(7) Disassemble the manometers and save the tubes and their contents. (Number the tubes!) Obtain two ml sample of firefly lantern solution from the teacher. Take this and your manometer solutions into a dark room. Place 10 drop samples of the firefly lantern solution into four wells of a spot-plate and when your eyes have adjusted to the dark, a single drop of juice from each manometer tube into one of the wells. All light in the room should now be extinguished. Do any of your mixtures of juice and firefly solution glow in the dark?

A.3 Experiment: GLUCOSE METABOLISM BY BOILED YEAST JUICE

Repeat steps 1, 2 and 3 of Experiment A.2. Then divide the yeast juice equally between two test tubes. Place one in a boiling water bath for four minutes. Save this boiled juice in a refrigerator. Place the other half of the juice in dialysis tubing contained in a beaker of distilled water (500 ml water). Dialyze overnight in a refrigerator.

Now set up three manometers. All receive glucose and phosphate as indicated for manometer #4 in the last experiment. No. 1 receives 5 ml

CO_2 production should be greatest in #4, with considerable production in #3. With the teacher's help students may be able to calculate how many moles of CO_2 were produced. For this they will need to estimate (or measure) the volume(s) of their manometers. They will need to know the value of 1 atmosphere pressure in mm of water and the value of the universal gas constant in the appropriate units. If the reaction had been allowed to go to completion, between 2 and 3 moles of CO_2 would have been produced per mole of glucose, with the consumption of 2 moles of inorganic phosphate.

Firefly lantern solution: Add 25 ml of distilled H_2O to vial of powdered lantern extract. Mix well to make a cloudy solution. Use within a few hours.

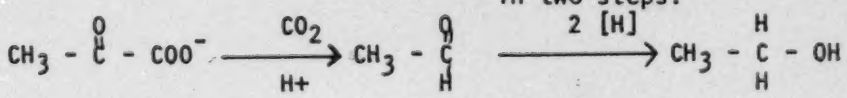
The juice from manometer #4 should glow most brightly, indicating the highest concentration of ATP, resulting from glucose fermentation.

Because A.3 repeats A.2, chemicals and materials are the same except for dialysis tubing. There are 3 conditions: boiled juice in which enzymes are denatured but small molecule cofactors are present and active, dialyzed juice containing active enzymes but little of the cofactors, and the mixture of the two (which should metabolize glucose).

A major dialyzable cofactor is ADP. Without this no glucose breakdown can occur.

of boiled yeast juice, No. 2 receives 5 ml of dialyzed yeast juice and No. 3 receives 2.5 ml of each, boiled and dialyzed yeast juices. Repeat steps 4, 5 and 6 of Exp. A.2 with these manometers. In which do you get CO_2 production?

The analysis of glucose breakdown by yeast juice and also by muscle-juice (the processes are almost identical) occupied biochemists for 40 years. Gradually, a step at a time, they were able to identify the individual steps in the reaction pathway (metabolic pathway) and in every case to isolate and purify the enzyme responsible. This pathway, called the Embden-Meyerhof-Parnas (EMP) pathway is basically the same in yeast, man, trees and every other living thing. This pathway is shown in Figure A.2 on the next page, leading from one molecule of glucose to two molecules of pyruvate. In fermenting yeast the pyruvate is converted to ethanol in two steps.



pyruvate acetaldehyde ethanol

In the muscles of an athlete running the 100 yard dash pyruvate is converted to lactate in one step.

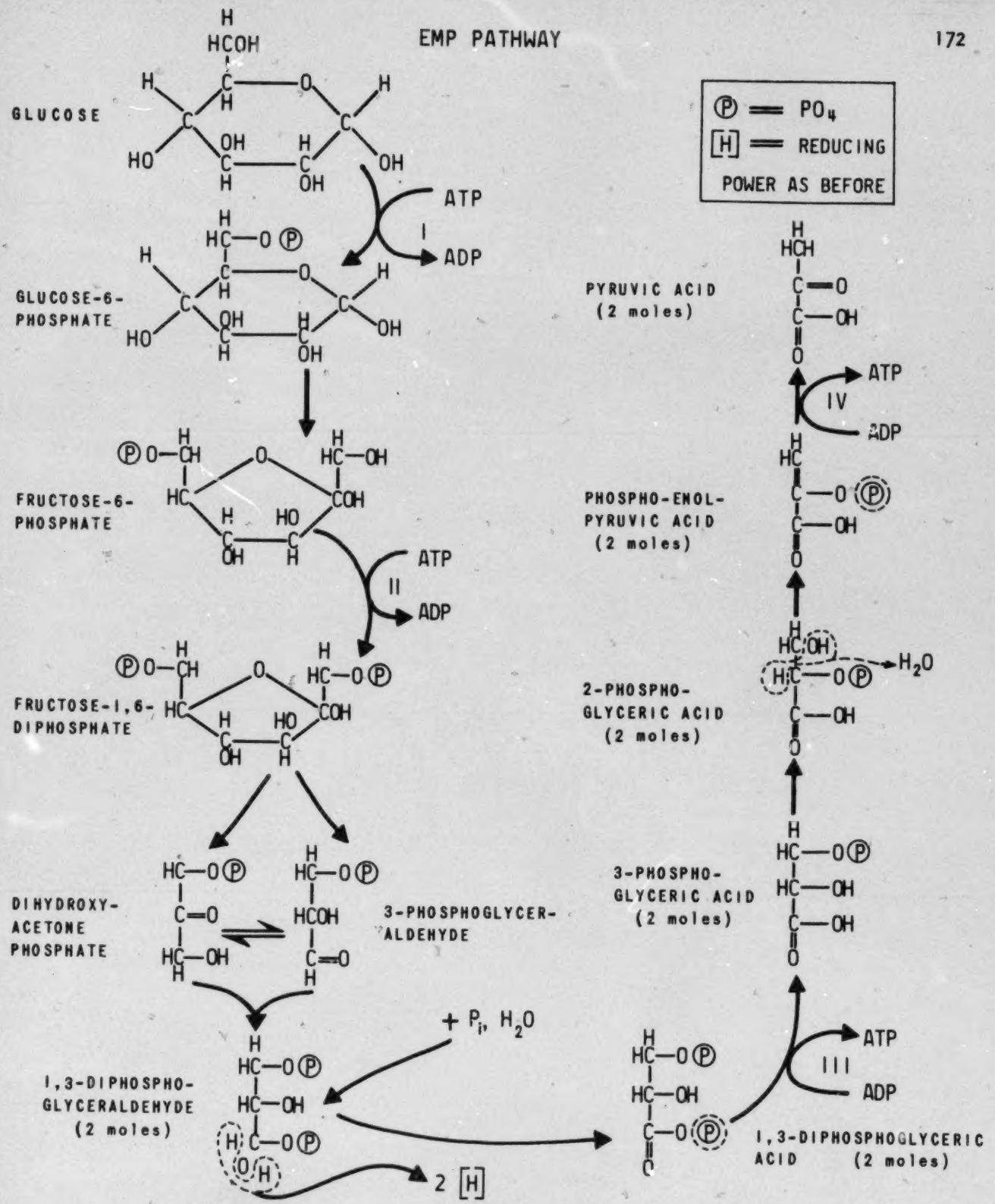
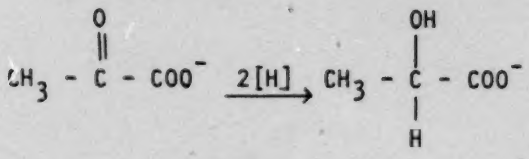

GLUCOSE
ATP
I
ADP
GLUCOSE-6-PHOSPHATE
FRUCTOSE-6-PHOSPHATE
ATP
II
ADP
FRUCTOSE-1,6-DIPHOSPHATE
DIHYDROXY-ACETONE PHOSPHATE
3-PHOSPHOGLYCER-ALDEHYDE
+ Pi, H2O
1,3-DIPHOSPHO-GLYCERALDEHYDE (2 moles)
2 [H]
Ⓟ = PO4
[H] = REDUCING POWER AS BEFORE
PYRUVIC ACID (2 moles)
ATP
IV
ADP
PHOSPHO-ENOL-PYRUVIC ACID (2 moles)
2-PHOSPHO-GLYCERIC ACID (2 moles)
H2O
3-PHOSPHO-GLYCERIC ACID (2 moles)
ATP
III
ADP
1,3-DIPHOSPHOGLYCERIC ACID (2 moles)

Figure A.2



pyruvate lactate

In both cases, but especially for the athlete, the temporary product, ethanol or lactate, is completely burned up by another pathway when O_2 becomes available. This is why you can run now and breathe bard later.

It is not important that you remember the details of this scheme. But some things you should see and understand. For example the importance of phosphate and how it is used. First you can see from the EMP diagram that the cell has to spend two molecules of ATP to convert glucose into the right chemical form (fructose -1, 6-diphosphate) for further reactions. Then it can earn, from further reactions, four molecules of ATP. Thus the net gain is two molecules of ATP from ADP and phosphate. (Do you see now why the yeast juice requires added phosphate to ferment glucose?)

This pathway can be run in the reverse direction with a few alterations. Thus it can be used to synthesize glucose and then starch from simpler molecules. If you remember 3-phosphoglyceric acid (PGA) as the initial product of

CO_2 fixation in photosynthesis, then running this reaction scheme backwards makes sense. It is undoubtedly how starch is made in plants. Starch is then stored energy which the plant uses in the dark and at other times when photosynthesis is impossible.

A couple of large questions might bother you at this point. For instance, how come yeast grows so much better with O_2 than without it? And how do we get from any of the chemicals in the EMP pathway to any and all of the amino acids and nucleotides? After all, these latter chemicals are the important ones in growth.

The answer to the first question is that there is still another pathway for the complete combustion of pyruvate when O_2 is available. This is called the tricarboxylic acid cycle (TCA cycle) and it produces, indirectly, 18 times as much ATP as the EMP pathway. For a diagrom of the TCA cycle see Figure A.3.

Also called the Krebs Cycle or Citric Acid Cycle.

Once again there are some important points to see:

1. The three carbon atoms in pyruvate are all converted to CO_2.
2. For every turn of the cycle one molecule of pyruvate is combusted and one molecule of oxaloacetate is regenerated. Obviously some

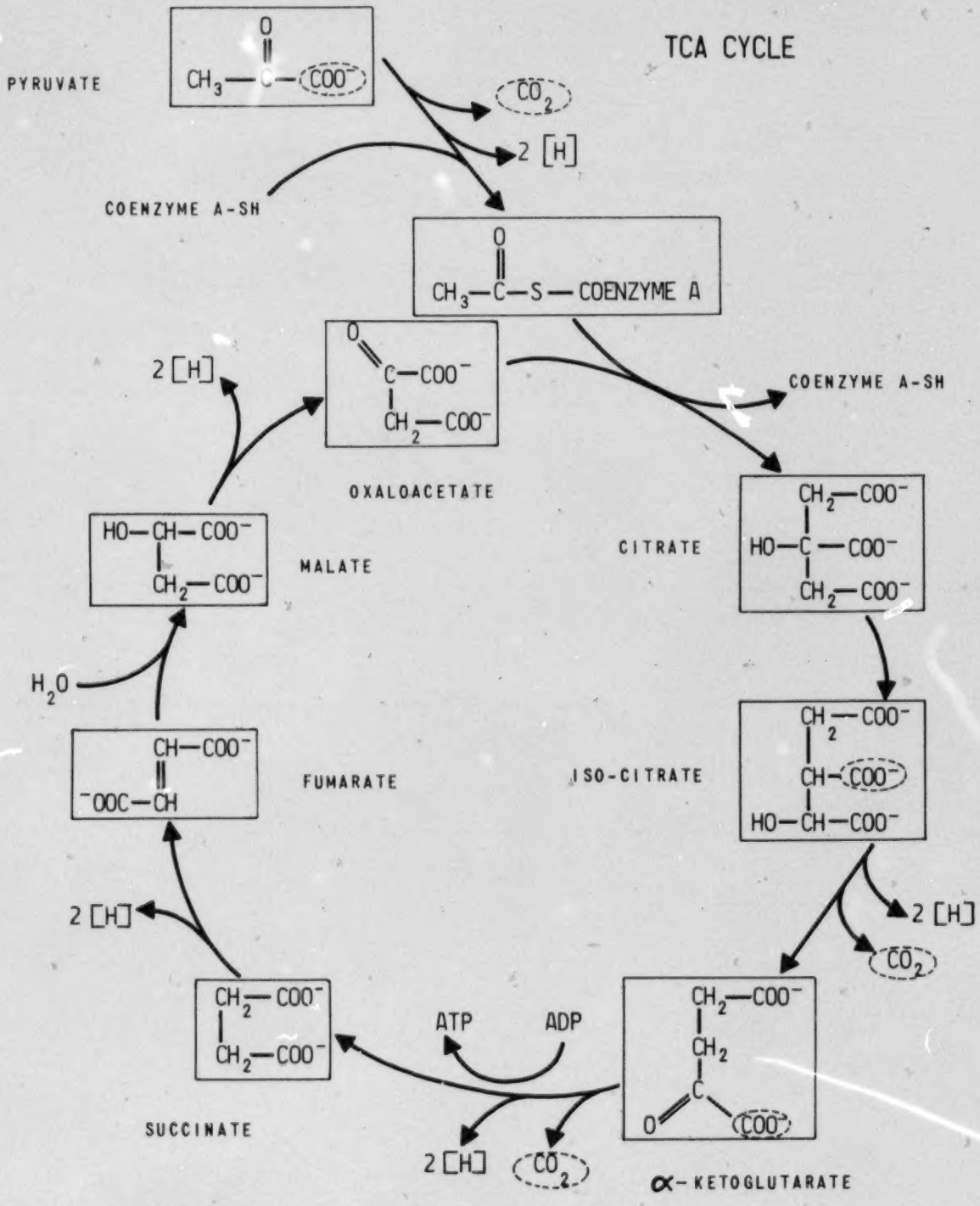


Figure A.3

CH_3-C-S-Coenzyme A is an activated form of acetate (acetic acid) analogous to other activated forms of carboxylic acids. This activation permits the reaction with oxaloacetate to proceed.

of the reactions are used to "get into position" for other reactions which actually produce usable energy. You may ask how all of the ATP's are produced? The answer is that the reducing power of [H] is used to do this. This is done in another pathway which is still largely a mystery. We do know that the reducing power is transferred step by step down a chain of stronger and stronger oxidizing agents until ultimately it is used to reduce O_2 to two molecules of H_2O. In some of these oxidation-reduction steps, ATP is produced. Overall this pathway makes 3 molecules of ATP for each pair of protons which make the trip.

The "electron transport chain."

In the electron transport system what is actually transferred down the chain is a pair of electrons. The protons "accompany" them, the ultimate reaction being:

$$\tfrac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$$

The second question referred to the formation of amino acids, nucleotides and other essential compounds. It has been shown that the two pathways just considered are the "core" of the metabolism of almost every living thing. There are "side streets" leading off at various points to all of the amino acids and other monomers which the cell needs. If we schematize the EMP and TCA pathways, some of the side streets can be visualized.

What is really amazing is the economy in the number of steps used by living things to produce all the chemicals they need. The whole

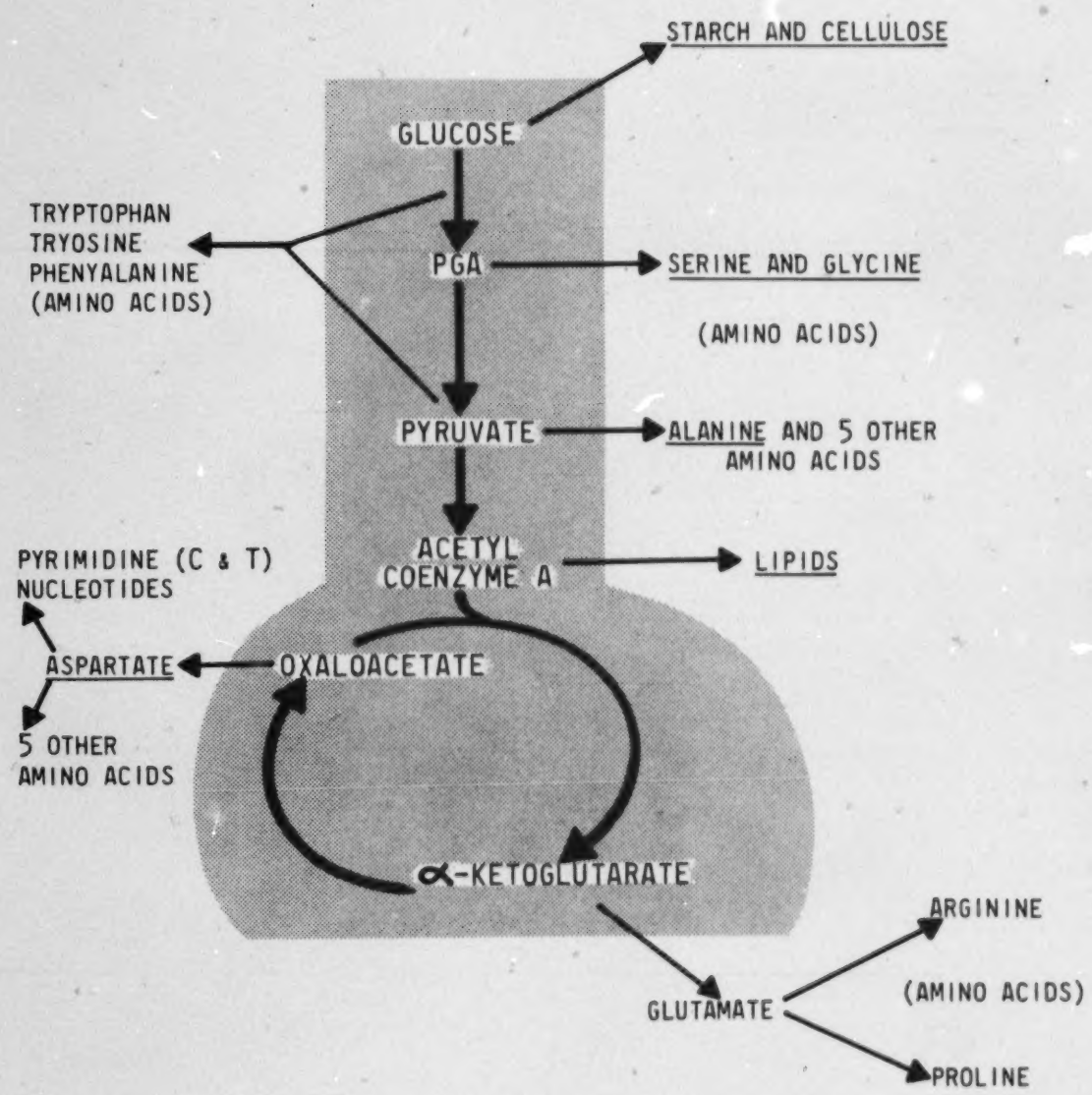

STARCH AND CELLULOSE
GLUCOSE
TRYPTOPHAN
TRYOSINE
PHENYALANINE
(AMINO ACIDS)
PGA
SERINE AND GLYCINE
(AMINO ACIDS)
PYRUVATE
ALANINE AND 5 OTHER
AMINO ACIDS
PYRIMIDINE (C & T)
NUCLEOTIDES
ACETYL
COENZYME A
LIPIDS
ASPARTATE
OXALOACETATE
5 OTHER
AMINO ACIDS
α-KETOGLUTARATE
ARGININE
(AMINO ACIDS)
GLUTAMATE
PROLINE

Figure A.4

metabolic scheme is a little like the road map of a well-designed city. There are the freeways branching off into main streets branching off into side streets. The main pathways produce usable energy (ATP and reducing power) which are then consumed in the branch pathways. (See Figure A.4.)

Exercises for Home, Desk and Lab (HDL's)

(1) Proteolytic enzymes are capable of digesting what kind of polymers down to what kind of monomers?

(2) An enzyme in saliva can convert the starch polymer into what monomers?

(3) a. Under what two circumstances may glucose be degraded by yeast?

b. Write the balanced equation for each.

c. Which reaction appears to be the reverse of photosynthesis?

(4) a. When glucose is converted to pyruvate the reaction requires how many molecules of ATP?

(1) Proteolytic enzymes split proteins to amino acids.

(2) This enzyme converts starch to glucose.

(3) a. Glucose may be degraded by yeast either with or without the presence of oxygen. Aerobic degradation occurs in the presence of oxygen. Anaerobic degradation occurs in the absence of oxygen.

b. $C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6\ H_2O +$ *usable energy*

$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + CO_2 +$ *usable energy*

c. The aerobic degradation looks like photosynthesis run in reverse.

(4) a. Two molecules of ATP are required for the conversion of glucose to pyruvate.

b. Four molecules of ATP are produced when glucose is converted to pyruvate, which leaves the cell with a net increase of two molecules of ATP.

(5) a. This pathway is called the Embden-Meyerhof-Parnas pathway.

b. Yeast is able, in two steps, to convert it to ethanol. Man's muscles are able to convert it to lactate.

(6) Yeast is able to grow better in air because there is an additional pathway, called the tricarboxylic acid cycle, for the complete combustion of pyruvate when D_2 is available.

(7) a. 18 amino acids are shown as resulting from this scheme.

b. Tryptophan, tryosine, phenylalanine, arginine, proline, alanine, aerine and glycine.

(8) a. No.

b. Yes, the larger the amount of phosphate, the greater the rate of production of CO_2.

b. How many molecules of ATP are produced during this series of reactions?

(5) a. The metabolic pathway which is basically the same in yeast, man, trees and other living things is known by what name?

b. The pyruvate produced by this pathway can be converted by yeast and man to what?

(6) Why is yeast able to grow better in air than without air?

(7) In the schematized EMP and TCA pathways you are shown some of the side streets which lead to the production of amino acids needed by the body.

a. How many amino acids are shown to be produced?

b. List the names of specific amino acids shown in the scheme.

(8) a. Are whole, live yeast cells required to break down glucose?

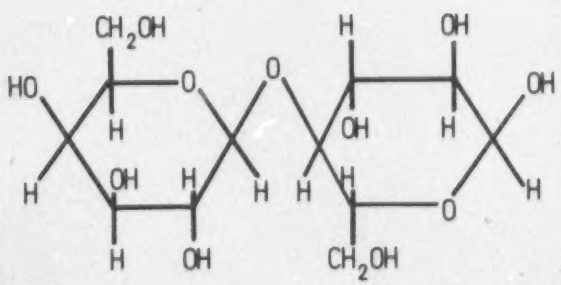
b. Does the amount of phosphate present in a solution affect the metabolism rate? Explain.

Chapter III: METABOLISM AND GENES

Many small molecules other than glucose can serve consumers as total sources of usable energy and of monomers. These include other kinds of sugars as well as alcohols, carboxylic acids and amino acids. Each of these potential foodstuffs requires special enzymes for its breakdown. These special catabolic enzymes are useless unless the particular substrate molecule is present in the environment. One would expect, therefore, that a special enzyme would not be produced unless its substrate were available. In fact, this is what is found for many consumer organisms.

Catabolic enzymes break down or convert foodstuff molecules into usable energy and at the same time synthesize the starting material for essential monomers.

The breakdown of lactose (the sugar found in milk) by the bacterium E. coli (Escherichia coli) is a good example of catabolism. Lactose is a disaccharide; that is, it is made up of two simpler sugar residues, one glucose and one galactose.



GALACTOSE RESIDUE

GLUCOSE RESIDUE

LACTOSE

Here galactose is in the β form. It is bonded via the one-carbon oxygen of galactose (in the β configuration) to the four position of glucose. Hence it is often called a β-galactoside.

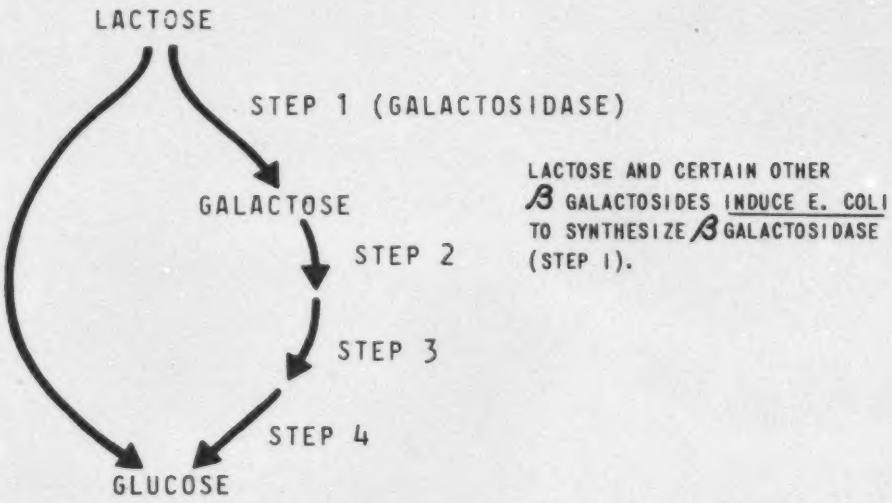
Mammals must perform the same hydrolysis of lactose in order to utilize it.

In order for E. coli to utilize lactose it must first hydrolyze or split lactose into its two monomeric components, galactose and glucose, utilizing a special catabolic enzyme.

$$\text{lactose} + H_2O \xrightarrow{\text{enzyme}} \text{galactose} + \text{glucose}.$$

The glucose can then be used directly via the EMP pathway, while the galactose must first be converted into glucose. This requires another three enzymes. Overall the total conversion of lactose to glucose is:

Lactose and certain other β galactosides induce E. coli to synthesize β galactosidase (step 1).



The enzyme that performs step 1 is called β-galactosidase. E. coli makes it only when presented with lactose or a similar β-galactoside. β-galactosidase is specific for the β-galactose half of the molecule and will therefore hydrolyze or split almost any molecule containing this sugar.

There are a number of important points in regard to this metabolic pathway. One is that E. coli makes only a tiny amount of β-galactosidase unless lactose or some other β-galactose is present. Thus this very simple organism has the ability to turn on and off one of its genes. Another is that β-galactosidase is the only pathway of lactose utilization available.

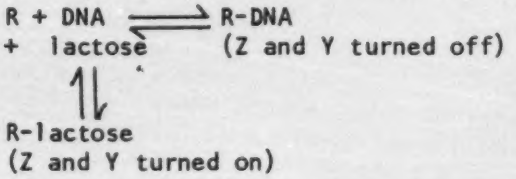
Actually there are two genes involved in lactose uptake and splitting. One, called the Z gene, specifies β-galactosidase and the other, called the Y gene, specifies the permease protein. This latter protein transports lactose across the cell membrane into the cell. Without it lactose cannot enter. Some mutants can make β-galactosidase but cannot eat lactose. These are permease-negative mutants, unable to pump lactose into the cell. Both of these genes (Z and Y) are turned on by lactose or some related galactoside in the environment. Another gene (the

Steps 2, 3, and 4 are catalyzed by a set of enzymes induced by galactose, not lactose. Both in E. coli and in humans a genetic defect causing loss of function of the enzyme catalyzing step three leads to a disease called galactosemia. For the bacteria this disease is fatal, and for human infants this defect leads to feeblemindedness if the infant is fed normal lactose-containing milk.

Each E. Coli cell can contain as many as 3000 molecules of β-galactosidase.

There are probably hundreds of its genes which E. Coli can turn on and off. The same enzyme is found in the lining of the human intestine. Why is it there?

i gene) is involved in this control of Z and Y function. The i gene specifies another protein, called the repressor, which specifically binds to a small region of the DNA near the Z gene, thereby preventing any messenger RNA synthesis for the Z or Y genes. The repressor (R) is a double-active-site enzymes. One site combines with the specific DNA segment, the other site with lactose. These two binding activities are mutually antagonistic such that when there is sufficient lactose around all of the repressor is "pulled" off the DNA.



The lactose genes Z and Y constitute what is called an inducible system, that is, a set of genes which are induced or turned on by the presence of the right chemical in the environment. Almost all energy-producing (catabolic) systems are inducible. Why does this make sense for the organism?

On the other hand the amino acid synthesizing pathways (sets of enzymes) are repressible. That is, the presence of the particular amino acid in the environment turns off the synthesis of the

These active sites are binding sites, not catalytic sites (such as when urease occupies one site, distorting the other binding site, i.e. pulling it out of shape).

Mutations in the i gene stop the synthesis of active repressor. Hence the Z and Y genes are turned on all of the time in such mutants. The repressor was only a hypothesis in the minds of geneticists until recently, when it was finally isolated and purified. This was a very difficult task as there are only tiny amounts of repressor--about 10 molecules per cell.

E. coli can eat about 50 different sugars, alcohols and organic acids. With a few exceptions all these compounds are metabolized by induced enzymes, that is, enzymes made only in the presence of the compound.

enzymes responsible for production of that amino acid. This means that E. coli will not make this particular amino acid from glucose as long as it can get it "free." Does this make sense?

This sort of control of gene activity takes place in all living things. For more complicated organisms it is a more complicated process but still essentially the same. Every cell in every organism is using only part of its genes at any time. When this control breaks down the consequences can be very bad. For example many scientists believe that cancer cells are cells which have lost the ability to control certain genes.

Some genes, such as those specifying the enzymes in the EMP and TCA pathways, are always turned on because the cell always needs these pathways. Nevertheless these genes are also controlled, since the cell may need more or less energy under differing conditions.

E. coli is probably not using more than 1/2 (about 1500) of its genes at any one time.